# MILLIMITRE WAVE QUASI-OPTICAL SIGNAL PROCESSING SYSTEMS

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# AN INVESTIGATION INTO THE FUNCTION OF SINGLE-NEURON ACTIVITY IN THE MESOACCUMBENS DOPAMINE SYSTEM OF THE RAT

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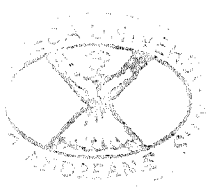

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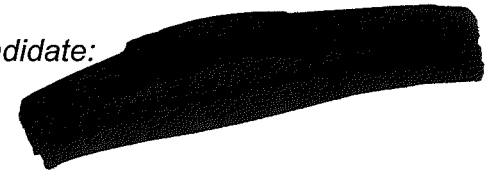
*Date:* June 1st 2005 *Signature of candidate:* 

## ABSTRACT

The mesoaccumbens dopamine system has been implicated in many basic psychological processes (e.g. "wanting" and "liking") and illnesses (e.g. addiction, depression, schizophrenia). However, the precise computational functions of nucleus accumbens and dopamine neurons within the system remain unknown. In this thesis, we test some of the current hypotheses regarding the function of this system using a behavioural neurophysiology approach in the rat. The first question we wanted to answer was whether nucleus accumbens neurons process reward-predictive stimuli (e.g. conditioned reinforcers) and reward delivery differently, since previous studies report equivocal findings. To do so, we trained thirsty rats to bar-press on a second-order schedule of saccharin reinforcement, within which the temporal pattern of rats' bar-pressing was reinforced by presentations of a conditioned reinforcer and primary reinforcer (reward). We found that nucleus accumbens neurons typically responded to these conditioned and primary reinforcers with opposite sign, which suggests they were processed differently. We were not sure whether responses to conditioned reinforcers encoded reward-prediction or facilitated a behavioural switch in the rat's behaviour. Indeed, since studies using a variety of experimental techniques have implicated the mesoaccumbens dopamine system in both reward prediction and behavioural switching, we sought to test whether neurons in the nucleus accumbens and dopamine-rich areas of the midbrain respond to outcome-associated stimuli to predict reward or switch behaviour. We found both sets of neurons predominantly did the former. Finally, to understand more about reward consummatory responses from both sets of neurons, we developed a rat behavioural task providing measures of reward "wanting" and "liking". In conclusion, on the basis of our data, the most parsimonious explanation for the function of the mesoaccumbens dopamine system is that it acts to modulate goal-seeking behaviour. Further research is required to identify the function of the interactions between nucleus accumbens and dopamine neurons during goal-seeking and goal consumption.

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# CHAPTER 1

# GENERAL INTRODUCTION

## INTRODUCTION TO THE MESOACCUMBENS DOPAMINE SYSTEM

### OVERVIEW

According to a popular biomedical search engine (PubMed) there are over 90,000 published articles with relevance to *dopamine* and almost 6,000 to *accumbens* and *dopamine*. Indeed, neural activity in this system has been correlated with a variety of fundamental psychological processes, such as humour, attraction, love, sex, hedonia, learning, attention, motivation, stress, eating and drinking. Perhaps more importantly, mesoaccumbens dopamine function has been implicated in many psychological illnesses including mood disturbance (e.g. depression, anxiety), addiction (drug, food, gambling), obsessions/compulsions (e.g. obsessive compulsive disorder, anorexia nervosa), psychosis (e.g. schizophrenia), and movement impairments (e.g. tics, attentional deficit hyperactivity disorder). However, the precise psychological correlates of this system and the neural mechanisms underpinning them are unclear.

We aim to identify and test some of the current hypotheses regarding the psychological function of the mesoaccumbens dopamine system. To do so, we will assess its functional anatomy, the behavioural consequences of manipulations to the system, and the neural correlates in the system during environmental or behavioural events. Throughout, we will describe hypotheses that have arisen within distinct research fields, typically formed on the basis of the experimental technique employed. Finally, we will identify which hypotheses and questions are available for us to test/answer using a behavioural neurophysiology approach in the rat.

*CORTICO-BASAL GANGLIA-THALAMIC-CORTICO LOOPS*

Neural circuit loops from cortex through basal ganglia (a collection of subcortical nuclei) to thalamus and back to cortex (cortico-basal ganglia-thalamic-cortico loops) have been implicated in processing emotional, cognitive and motor information (Haber 2003; Joel and Weiner 2000; Parent and Hazrati 1995; Voorn et al. 2004). Three main subdivisions of these loops have been proposed based on putative functions. Part of the 'limbic' loop, implicated in processing motivational information, includes projections from medial and orbital prefrontal cortex to ventromedial striatum. The 'cognitive' loop, suggested functions of which include working memory, attentional set-shifting and cognitive planning includes projections from dorsolateral prefrontal cortex to central caudate and putamen (central, dorsal striatum). Lastly, the 'motor' loop, which is thought to participate in movement initiation, is partly comprised of projections from caudal premotor, pre-supplementary motor and cingulate motor cortical neurons to dorsolateral striatum.

Consequently, it has been suggested that there is a functional gradient of motivational-cognitive-movement functions across ventromedial-central-dorsolateral areas of the striatum, reflecting functionally segregated cortical inputs (see Figure 1.1; Voorn et al. 2004). Inputs remain largely anatomically segregated within loops through cortical-thalamic, thalamic-striatal, striatal-pallidal, striatal-substantia nigra pars reticulata and thalamic-cortical projections. It should be noted that information does not flow one-way around the loop since connections within the basal ganglia and between cortex and thalamus are reciprocal. Although information can remain segregated within these loops, recent findings suggest that it can also pass from one loop to the other (Haber 2003; Joel and Weiner 2000). Possible mechanisms facilitating this integration include crossover of axons and dendrites, convergence of neurons at the edge of loops and non-reciprocal feed-forward connections.

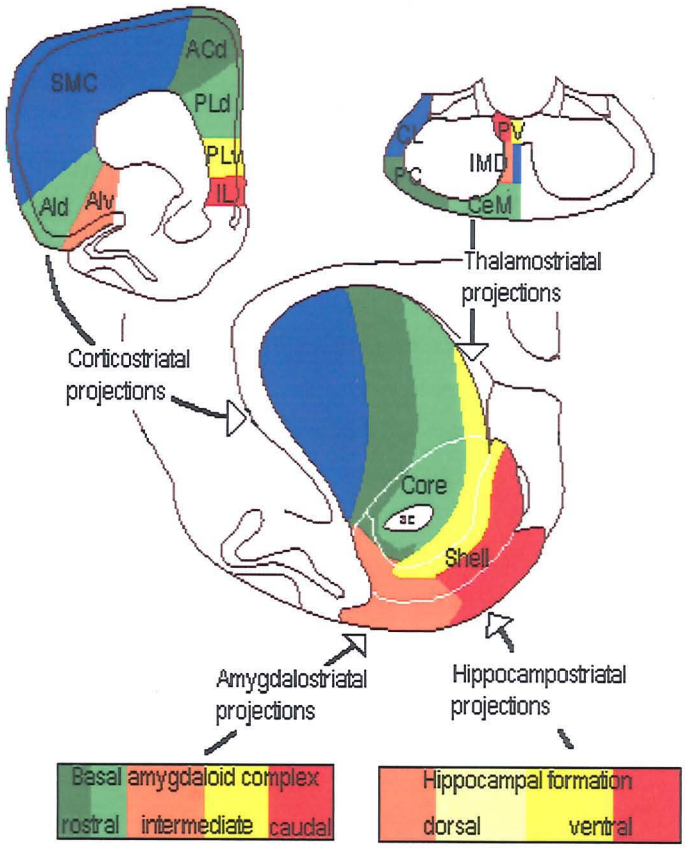



*Figure 1.1* Cortical, thalamic, amygdaloid and hippocampal inputs to the striatum. Coloured gradient of red-green-blue represents limbic-cognitive-motor information processing, respectively across a ventromedial-central-dorsolateral gradient of the striatum. Part of this distinction includes the shell (ventromedial) and core (dorsolateral) portions of the nucleus accumbens. *Abbreviations:* ac, anterior commissure; Acd, dorsal anterior cingulate cortex; Ald, dorsal agranular insular cortex; Alv, ventral agranular cortex; CeM, central medial thalamic nucleus; CL, central lateral thalamic nucleus; IL, infralimbic cortex; IMD, intermediodorsal thalamic nucleus; MD, mediodorsal thalamic nucleus; PC, paracentral thalamic nucleus; PFC, prefrontal cortex; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; PV, paraventricular thalamic nucleus; SMC, sensiromotor cortex. Modified from Vroon et al. (2004).

*NUCLEUS ACCUMBENS*

The 'limbic' striatum described above, also known as the ventral striatum, is comprised of the nucleus accumbens, ventral parts of caudate and putamen and striatal parts of the olfactory tubercle (Joel and Weiner 2000). The nucleus

accumbens can be further subdivided into core and shell areas based on differential inputs and outputs (see Figure 1.1) (Voorn et al. 2004; Zahm 2000). Inputs to the core are most similar to inputs to the central areas of the striatum whereas the shell receives afferents from the most 'limbic' cortices (Vroom et al. 2004). Outputs from the shell project to 'limbic' ventromedial ventral pallidum, and areas implicated in autonomic responses such as lateral hypothalamus and brainstem. However, output from the core is more akin to the rest of the striatum, with projections to the dorsolateral ventral pallidum and then subsequently to substantia nigra pars reticulata (Voorn et al. 2004; Zahm 2000). As well as cortical inputs, the ventral striatum receives input from the amygdala and hippocampus, areas also implicated in motivation, as well as in learning and memory.

*MIDBRAIN DOPAMINE AREAS*

Midbrain dopamine neurons are found in four main areas: the ventral tegmental area (a ventral medial midbrain area), the substantia nigra pars compacta (a thin structure lateral to the ventral tegmental area that spans ventromedially to dorsolaterally), the retrorubral area (a group of neurons caudal and dorsal to the other dopamine sites) and the substantia nigra pars reticulata (the few dopamine neurons here are present from ventrally extending dendrites from the dorsal layer of substantia nigra pars compacta cells) (Joel and Weiner 2000). Inputs to these areas include the amygdala, striatum, pallidal complex, subthalamic nucleus, dorsal raphe and the pedunculopontine nucleus (Schultz 1998).

The fibres projecting from dopamine neurons fall into one of three component systems: the mesostriatal (neurons in the substantia nigra pars compacta and a portion of ventral tegmental area that predominately project to dorsal striatum), the mesocortical (neurons mostly from the ventral tegmental area and dorsal and medial parts of the substantia nigra project to medial prefrontal, anterior cingulate and suprahinal cortices) and the mesolimbic (neurons in the ventral tegmental area and some parts of substantia nigra and retrorubral fields project to nucleus

accumbens, olfactory tubercle, amydala, bed nucleus of stria terminalis, lateral septal area and lateral hypothalamus) (Gardner and Ashby 2000). The focus in this thesis will be the mesolimbic dopamine pathway and in particular the reciprocal connections between dopamine and nucleus accumbens neurons (termed the 'mesoaccumbens dopamine system').

*RECIPROCAL CONNECTIONS BETWEEN DOPAMINE AND NUCLEUS ACCUMBENS NEURONS*

In the rat, nucleus accumbens neurons have reciprocal and feed-forward connections with midbrain dopamine neurons. The nucleus accumbens receives dopamine input primarily from the ventral tegmental area and projects back reciprocally. Additionally, nucleus accumbens neurons innervate dopamine neurons in the substantia nigra pars compacta. These dopamine neurons project to both 'cognitive' and 'motor' subdivisions of the dorsal striatum. However, most neurons in the 'cognitive' and 'motor' striatum do not project to dopamine-rich areas, but instead to substantia nigra pars reticulata neurons which use gamma-aminobutyric acid (GABA) as their neurotransmitter (Joel and Weiner 2000). This arrangement results in 'limbic' striatum influencing more dopamine neurons than they are influenced by, and 'cognitive'/'motor' striatum being influenced by more dopamine neurons than they influence (Haber et al. 2000; Joel and Weiner 2000).

In the primate (and possibly in the rat), this arrangement has been described as an upward spiral of inverse dorsal-ventral connections between midbrain dopamine regions and the striatum. Thus, shell nucleus accumbens neurons (ventromedial) have reciprocal connections with dorsal dopamine sites *and* make feed-forward non-reciprocal projections to more ventrolaterally placed dopamine neurons. Neurons here make reciprocal connections with neurons in the nucleus accumbens core (which is located dorsolaterally to the shell). Some core neurons additionally make non-reciprocal projections to dopamine neurons in even more ventral and lateral sites. This inverse dorsal-ventral upward spiral goes from

ventromedial to dorsolateral striatum connecting to dorsomedial to ventrolateral dopamine sites (see Figure 1.2; Haber et al. 2000).

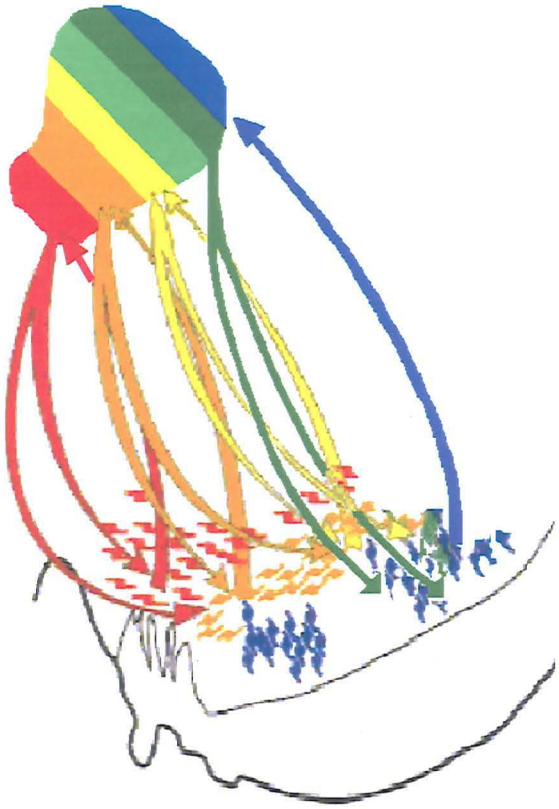


*Figure 1.2* Ascending inverse dorsal-ventral spiral between midbrain dopamine neurons (bottom) and striatal neurons (top, cross-section). Red-green-blue gradient represents limbic-cognitive-motor information. Note that striatal projections reciprocate a dopamine projection and feed-forward to dopamine neurons placed in more ventrolateral locations. Modified from Haber et al. (2000).

*MICROSTRUCTURE OF DOPAMINE AND STRIATAL SYNAPSES*

Most (~95%) striatal neurons are 'medium spiny' projection neurons releasing GABA (which inhibits the post-synaptic neuron), with the remaining neurons comprised of different types of interneurons (Kemp and Powell 1971; Tepper et al. 2004). It has been proposed that these projections can directly inhibit dopamine neurons or indirectly excite dopamine neurons. The latter situation can occur through disinhibition (inhibition of inhibitory projecting neurons) of GABA-releasing substantia nigra pars reticulata neurons or GABA-releasing interneurons in the ventral tegmental area that project to dopamine neurons. Medium spiny neurons have approximately 20-60 dendrites in a sphere around

the soma (Wilson 1995). Uniquely, each dendrite is covered in approximately 500 spines, onto which cortical neurons, which release the fast-acting neurotransmitter, glutamate, and dopamine neurons often synapse (the former at the head of the spine, the latter at the neck; see Figure 1.3) (Mink 1996; Smith and Bolam 1990; Wilson 1995).

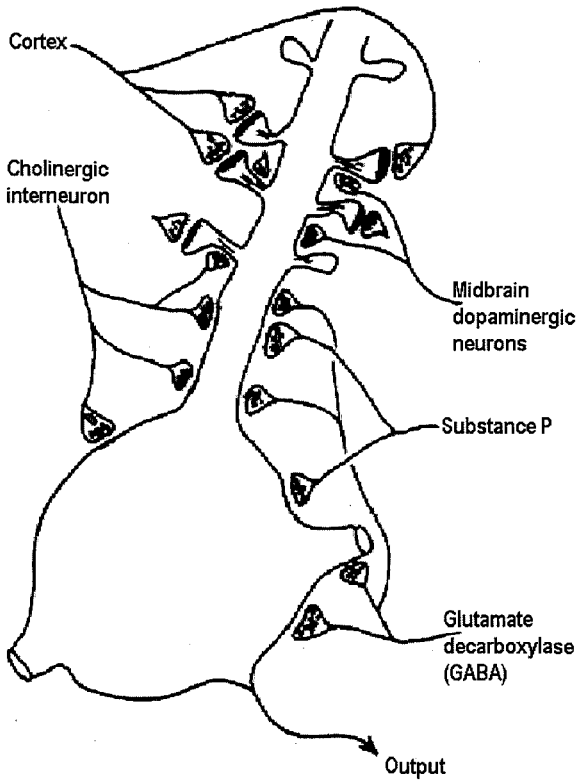



*Figure 1.3* Schematic of a medium spiny neuron. Note the appearance of spines from dendrites onto which dopamine and cortical neurons synapse at the neck and head, respectively. Modified from Smith and Bolam (1990).

The effects of dopamine on these synapses are not fully understood due to the difficulty in assessing intracellular events in the awake, behaving animal. However, a current popular hypothesis is that dopamine enhances the contrast between strong bursts of excitation coming into the neuron, e.g. from cortical neurons, and weak activations (Nicola et al. 2004a). Thus, dopamine inputs might enhance the signal:noise ratio by 'dampening' the activity of most neurons, whilst enhancing strong cortically-evoked glutamatergic excitations. Additionally, coincident cortical stimulation and dopamine release can potentiate post-synaptic

potentials from corticostriatal synapses, which has been proposed to be a potential mechanism of reinforcement learning (Schultz 1998; Wickens et al. 2003). Indeed, it has been demonstrated that the degree of this potentiation was correlated with the time rats took to learn to bar-press to receive 'rewarding' stimulation to its own substantia nigra, presumed to release dopamine into corticostriatal synapses (Reynolds et al. 2001).

## MANIPULATIONS OF THE MESOACCUMBENS DOPAMINE SYSTEM

### DOPAMINE MANIPULATIONS

#### *RESPONSE SELECTION HYPOTHESIS*

Lyon and Robbins theorised that administration of increasing doses of amphetamine (an indirect dopamine agonist) increases the repetition rate of all behaviours resulting in the organism producing increasingly shorter-lasting behaviours at an increased rate across fewer behavioural categories (Lyon and Robbins 1975). For example, the authors argued that administration of a low dose of amphetamine increases the rate of behaviours with complex chains, e.g. eating or grooming. At moderate doses these long chains of behaviour are unable to be produced or completed and short-duration or incomplete behaviours dominate e.g. rearing, turning. At even higher doses very few behaviours are made and responses are stereotyped e.g. repetitive lateral head movements, licking or biting. Eventually, at extreme doses no behaviours are present, since over-activation means even the shortest behaviour is unable to be completed. More recently, it has been proposed that the striatum constructs a 'response set', whereby it places a relative probability weighting on the possible responses to be selected. Amphetamine (and dopamine) might enhance these probability weightings, which at high doses would cause the current dominant behaviour to become even more dominant (Brown et al. 1996; Robbins and Brown 1990).

#### *BEHAVIOURAL SWITCHING HYPOTHESIS*

A possible consequence of dopamine-induced enhancement of response probability weightings is that at lower doses more response probabilities reach a threshold to be selected, resulting in increased switching between behaviours. Indeed, it has been found that low and medium doses of amphetamine administration increased switching between competing behaviours that were equally effortful and rewarding (Evenden 2002; Evenden and Robbins 1983b; Robbins and Watson 1981). Similarly, lesions of mesoaccumbens dopamine

neurons reduced switching between competing behaviours such as displacement drinking behaviour and reactivity to novel stimuli in hungry rats (Evenden and Carli 1985; Robbins and Koob 1980). Moreover, microinjections of amphetamine into the nucleus accumbens decreased the duration of eating bouts but increased competing behaviours such as locomotion, whereas haloperidol (a dopamine receptor ('$D_2$-like') antagonist) increased eating and decreased competing behaviours such as drinking (Bakshi and Kelley 1991a, b). Modifications in behavioural switching have also been reported in aversive situations. Within a forced swim task microinjections of amphetamine into the ventral striatum increased switching to cue-directed behaviours such as scanning of the swimming bath or detecting a safety rope (van den Bos and Cools 2003).

*ANHEDONIA HYPOTHESIS*

Alternatively, the anhedonia hypothesis states that dopamine receptor antagonists blunt the hedonic impact of rewards and their associated stimuli rather than cause motor impairments, *per se* (Wise 1982). Evidence cited in support of this hypothesis includes findings that these drugs increased the threshold of rewarding electrical brain stimulation, attenuated operant responding for natural rewards and reduced maintenance of eating bouts. However, many of these studies used behavioural measures of operant responses or behavioural choice responses making it difficult to dissociate putative motor *versus* hedonic impairments.

*BEHAVIOURAL ACTIVATION HYPOTHESIS*

In opposition to the anhedonia hypothesis, Salamone et al. (Salamone et al. 1997) argue that dopamine depletions do not reduce reward motivation or pleasure since depleted rats eat and drink freely available food and water to equivalent levels, or more, as control rats (Bakshi and Kelley 1991a; Evenden and Carli 1985; Robbins and Koob 1980; Salamone et al. 1993). Instead, they propose that dopamine depleted rats are impaired when effort is required to gain reward. For example, dopamine depletion in reward-choice tasks reversed

normal preferences to bar press for high-quality reward over consuming freely available poor-quality reward (Cousins and Salamone 1994; Cousins et al. 1993) and to choose to climb over a barrier for four food pellets *versus* facing no barrier and obtaining two food pellets (Salamone et al. 1994). Furthermore, depleted rats *can* respond normally when a low number of responses (1-5) are required to gain reward (Salamone et al. 2001). However, in this study depleted rats were impaired by further increases in the number of responses required to gain reward. These impairments were unlikely to be due to the number of responses *per reward* (Salamone et al. 2001) or the force of response required (Ishiwari et al. 2004), suggesting that rats were unable to reach an absolute level of effort required to gain reward. Salamone et al. propose that dopamine depletion in the nucleus accumbens impairs behavioural activation processes related to instrumental responding, making the organism less invigorated by the presence of conditioned stimuli or reward, and less able to respond effortfully to deal with work-related costs (e.g. barriers, bar presses) to gain reward or avoid punishment (Salamone and Correa 2002; Salamone et al. 2003).

*INCENTIVE SALIENCE HYPOTHESIS*

Another line of evidence against the anhedonia hypothesis comes from assessment of hedonic facial reactions by rats to sweet and/or bitter tasting solutions. Rats with lesions to all striatal dopamine neurons or enhanced dopamine neurotransmission in the nucleus accumbens by amphetamine microinjections had normal hedonic facial reactions to the taste of sucrose and quinine. In contrast, combined lesions of the lateral hypothalamus and ventral pallidum, or lesions of the basal forebrain, decreased positive and increased negative affective reactions, whereas opioid manipulations enhanced positive hedonic reactions (Berridge and Robinson 1998; Wyvell and Berridge 2000). Importantly, although dopamine-lesioned rats showed normal hedonic reactions, they had to be artificially kept alive by intragastric intubation because they were aphagic and adipsic. Berridge and Robinson (1998) hypothesise that dopamine transforms "liked" stimuli that bring an organism hedonic pleasure, e.g. reward,

into incentive salient stimuli, stimuli that are "wanted" and elicit attention. In this theory, dopamine is not necessary for associative learning (discussed below) although it can transform hedonic-predictive stimuli into incentive stimuli that, like reward, become wanted and attractive to the organism.

Indeed, manipulations in dopamine neurotransmission can alter the effects of incentive salient stimuli on behaviour. For instance, microinjections of amphetamine into the nucleus accumbens increased responding on a lever, which in training was predictive of reward, but only during presentation of a conditioned stimulus (a reward-associated stimulus) (Wyvell and Berridge 2000). This effect could not have been caused by amphetamine changing the hedonic experience of reward since reward was never delivered during amphetamine testing. Response enhancement was also not attributable to increases in general behavioural activation since amphetamine only caused slight increases in responding in the absence of conditioned stimulus presentation. Similar effects have been reported previously in a different task where amphetamine potentiated the effects of a conditioned stimulus as a reinforcer of a new response (conditioned reinforcer) (Taylor and Robbins 1984). Additionally, it has been demonstrated that 'hyperdopaminergic' mice bred without the dopamine transporter gene (resulting in increased extracellular dopamine) displayed increased "wanting" for sucrose reward, as measured on a runway test, but had normal hedonic facial reactions to sucrose (Pecina et al. 2003). Moreover, nucleus accumbens dopamine lesions reduced the effects of conditioned stimuli to elicit approach (Parkinson et al. 2002) and administration of dopamine antagonists attenuated the potentiating effects of conditioned stimuli on operant responding (Dickinson et al. 2000) and on their ability to elicit goal-directed responses (Wakabayashi et al. 2004).

*INCENTIVE SALIENCE OR BEHAVIOURAL ACTIVATION?*

The incentive salience hypothesis implicates dopamine function in motivation which correspondingly changes behavioural activation, whereas Salamone et al.

propose dopamine only mediates behavioural activation (Salamone and Correa 2002). Salamone et al. argue that lesioned rats have unimpaired motivation since they eat and drink as normal when reward is freely available. Counter to this, Berridge and Robinson (1998) assert that lesions to all of dopamine inputs to striatum, not just mesoaccumbens dopamine neurons, are required before the loss of reward "wanting" causes rats to be aphagic or adipsic. Indeed, these results can be explained by incentive salience (encoded by dopamine) having different functions in the nucleus accumbens and dorsal striatum. It has been previously been proposed that dopamine might select complex instrumental responses via the nucleus accumbens and stimulus-response habits via the dorsal striatum (Robbins and Everitt 1992, 2002). Therefore, lesions to mesoaccumbens dopamine might spare habit behaviours like consuming food. Consequently, dopamine probably serves an incentive salience function, which in the striatum can change behavioural activation.

*LEARNING HYPOTHESIS*

In dopamine lesioned rats, affective facial reactions (lateral tongue protrusions, rhythmic tongue protrusions or paw licks) changed to aversive (gapes, chin rubs, face washing, forelimb flails, paw tread, locomotion) when sucrose was paired with an aversive stimulus, lithium chloride, suggesting that striatal dopamine is not necessary to learn aversive associations (Berridge and Robinson 1998). Moreover, there is recent evidence that dopamine is not required for *reward* associative learning. 'Dopamine-deficient' mice, bred without the tyrosine hydroxylase (an intracellular enzyme used in the synthesis of dopamine) gene in dopamine neurons, were given caffeine (an adenosine receptor antagonist) to stimulate performance in a T-maze task. These rats were able to learn to choose the rewarded arm signalled by the sight of reward and contextual cues (Robinson et al. 2005). It has been argued (Berridge 2005) that it is unlikely mice were able to learn this task by caffeine acting in the same way as dopamine since caffeine fails to activate immediate early gene expression of c-Fos in the dorsal striatum of dopamine-deficient mice (Kim and Palmiter 2003). Additionally, it has been

shown that dopamine-deficient mice can learn to drink from a drinking spout to gain sucrose reward, which requires hedonic and reward learning processes (Cannon and Palmiter 2003).

However, it remains a possibility that dopamine contributes to associative learning, without being required. Indeed, rats with nucleus accumbens dopamine lesions made fewer conditioned stimulus approaches when lesions were made pre- *versus* post-task acquisition (Parkinson et al. 2002). However, as the authors discussed, it is possible that throughout learning rats began to approach conditioned stimuli in a habitual manner and neural processing had shifted to the dorsal striatum. Therefore, the lesion may have had reduced effects after task acquisition for reasons other than learning. However, it has recently been demonstrated that systemic independent administrations of $D_1$ or $D_2$ receptor antagonists impaired and improved learning of conditioned stimulus-reward associations, respectively (Eyny and Horvitz 2003). This was evidenced by changes in the number of food magazine entries during conditioned stimulus presentation on the day after learning, in the absence of drug testing and reward delivery. Similarly, it has been demonstrated that microinjections of $D_1$, but not $D_2$, receptor antagonists into the nucleus accumbens shell, but not core, impaired the ability for rats to learn aversive associations as measured by consumption rates and taste reactivity (Fenu et al. 2001).

Moreover, it has been argued that administration of dopamine antagonists can block the acquisition of conditioned place preference for psychostimulants, impair acquisition of drug self-administration and cause a burst of responses in well-trained subjects similar to the initial burst of responses found when reward is omitted (Di Chiara 2002). Finally, simultaneous microinjections of very low doses of a $D_1$ receptor antagonist and a selective competitive antagonist of the glutamate receptor *N*-methyl-*D*-aspartate into the nucleus accumbens core area slowed learning of a bar press response to gain food reward, yet did not affect locomotor activity or feeding rates (Smith-Roe and Kelley 2000). In summary,

lesion and gene knockout manipulations suggest that dopamine is not *required* for simple associative reward learning (Robinson et al. 2005), although psychopharmacological manipulations suggest that dopamine *contributes* to instrumental and associative learning (Eyny and Horvitz 2003; Fenu et al. 2001; Parkinson et al. 2002; Smith-Roe and Kelley 2000).

*SYNTHESIS: DOPAMINE CONTRIBUTES TO INCENTIVE SALIENCE AND LEARNING*

Manipulations of dopamine neurotransmission in the striatum has different effects within different striatal areas (Bakshi and Kelley 1991a, b; Robbins and Everitt 1992). This might account for the sparing of simple instrumental behaviours following lesions to nucleus accumbens dopamine fibres found by Salamone et al. (Salamone and Correa 2002). Although striatal dopamine does not seem to be required for associative learning it does seem to contribute, since dopamine depletion (in particular $D_1$ receptor antagonism) can impair associative and instrumental learning. Therefore, it seems most parsimonious to conclude from the above studies that dopamine provides a motivational salience signal. As part of accumbens-dopamine-dorsal striatum spiralling loops (Haber et al. 2000) this could facilitate learning and bias selection of adaptive, complex, instrumental behaviours in the nucleus accumbens and of simpler habitual responses that are often required during consummatory behaviour in the dorsal striatum.

*BEHAVIOURAL SWITCHING*

Nucleus accumbens lesioned rats produce similar behavioural switching impairments as dopamine depleted rats. Thus, lesions can reduce switching of behavioural strategies and/or attention to relevant stimuli within instrumental tasks to gain reward and can reduce switching to *stop* responding for reward when it is omitted (extinction), or when response requirements become excessive (Bowman and Brown 1998; Reading and Dunnett 1991; Reading et al. 1991).

*ENHANCEMENT OF BEHAVIOURAL ACTIVATION BY INCENTIVE SALIENT CUES*

Lesions to the nucleus accumbens core seem to decrease the attraction of incentive stimuli. Thus, core lesions decreased the number of approaches made to a conditioned stimulus (Parkinson et al. 2000b) and impaired the acquisition of conditioned place preference for sucrose reward, a phenomenon where rats spend more time in an environment previously associated with reward *versus* an unpaired environment (Everitt et al. 1991). Moreover, nucleus accumbens lesions abolished the stimulatory (Corbit et al. 2001; de Borchgrave et al. 2002; Hall et al. 2001) and reinforcing (Everitt et al. 1989; Hutcheson et al. 2001) effects of conditioned stimuli on instrumental responding (these effects were predominantly attributed to the core not shell, although the opposite pattern was found by Corbit et al. (2001)). Core lesions have also impaired learning that a neutral stimulus is associated with reward through its pairing with a previously conditioned stimulus (Setlow et al. 2002).

However, core lesioned rats seem able to learn to approach the reward magazine upon conditioned stimulus presentation (Corbit et al. 2001; de Borchgrave et al. 2002; Hall et al. 2001). Although it has been found that core lesioned rats reduced approaches to reward during conditioned stimulus presentation, this result was confounded since the conditioned stimulus was presented in the same location as reward (Parkinson et al. 1999). These results

suggest that the nucleus accumbens (probably the core) is required to use incentive salient stimuli to activate behaviour. However, it is not *required* to learn conditioned stimulus-reward associations.

*INSTRUMENTAL LEARNING*

Similarly to dopamine lesions (Salamone and Correa 2002), nucleus accumbens lesions do *not* abolish the previously learnt ability by rats to respond for immediately delivered reward (instrumental responding) (Balleine and Killcross 1994; Cardinal and Cheung 2005; Hutcheson et al. 2001; Reading et al. 1991). Moreover, lesioned rats can *learn* a new response that immediately delivers reward (instrumental conditioning) (Alderson et al. 2001; Cardinal and Cheung 2005; Corbit et al. 2001; de Borchgrave et al. 2002), and can adapt responses to changes in the outcome value or contingency between action and outcome (Alderson et al. 2001; Balleine and Killcross 1994; de Borchgrave et al. 2002) (although Corbit et al. (2001) did report that core lesioned rats produced responses rates that were equally low for devalued *versus* non-devalued reward).

However, responding during instrumental conditioning in lesioned rats is usually at a lower rate and can be abolished by the introduction of a delay between the action and outcome (Cardinal and Cheung 2005). These data suggest that rats are impaired at learning rather than performing actions to gain an outcome. Indeed, microinjections of a glutamate receptor antagonist, *N*-methyl-*D*-aspartate, into the nucleus accumbens core slowed learning of an instrumental response (although rats were able to produce responses) (Kelley et al. 1997), and learning and performance within a radial arm maze (Smith-Roe et al. 1999). Strikingly, inhibition of protein synthesis within the nucleus accumbens (protein synthesis contributes to synaptic plasticity) *after* behavioural training impaired consolidation of an instrumental response (Hernandez et al. 2002). Importantly, these effects were unconfounded by possible motor impairments or drug side effects within the task. Thus, the nucleus accumbens contributes to learning instrumental responses and maintaining delays between actions and outcomes.

*CONSUMMATORY BEHAVIOURS AND HEDONIC ATTRIBUTION*

Nucleus accumbens dopamine does not affect consummatory responses (Salamone and Correa 2002). Although nucleus accumbens lesions do not change food consumption rates, hoarding of food can be reduced (Stern and Passingham 1994; Whishaw and Kornelsen 1993). However, the nucleus accumbens can *contribute* to consummatory responses since microinfusions of the glutamate receptor antagonist, AMPA, and a GABA agonist into the medial shell area of the nucleus accumbens increased feeding for food and sucrose, although not water (Kelley 2004). In fact, it has been demonstrated that microinjections of these compounds into the *rostral* medial shell induces motivated behaviours with a positive valence, such as feeding, conditioned place preference to sucrose, and positive hedonic reactions to sucrose taste, whereas injections into the *caudal* shell induces motivated behaviours with a negative valence such as defensive treading, conditioned place avoidance to quinine, and negative hedonic reactions to sucrose or quinine tastes (Reynolds and Berridge 2003, 2002). This was hypothesised to reflect a rostro-caudal gradient within the nucleus accumbens shell for the initiation of motivated behaviours and hedonic reactions with a positive-to-negative motivational valence.

It has also been found that opioid agonism within the nucleus accumbens can enhance intake of palatable foods. Thus, microinjections of D-Ala2, Nme-Phe4, Glyol5-enkephalin (DAMGO; a mu opioid receptor agonist) increased rats' intake of, and motivation to bar-press for, sucrose, salt and saccharin, although not water (Zhang et al. 2003; Zhang and Kelley 2002), and enhanced choices of fatty *versus* carbohydrate foods (Zhang et al. 1998). Additionally, systemic administration of morphine, an opioid agonist enhanced hedonic reactions to tasting sucrose (Rideout and Parker 1996). Kelley (2004) argues that enhancement of feeding can be induced by microinjections of opioid agonists throughout the nucleus accumbens and dorsal striatum. Further, she postulates that manipulations to opioid neurotransmission in the nucleus accumbens affect

processing of *'tasty'* foods e.g. sweet, salt, whereas GABA/glutamate manipulations affect *calorific* feeding (which would not include salt or saccharin). Moreover, GABA/glutamate stimulatory effects are limited to the medial shell of the nucleus accumbens, a region that contains projections to lateral hypothalamus, suggesting a specific role for these neurons in feeding. Conversely, opioid-induced stimulation of feeding throughout the nucleus accumbens might encode hedonic "liking", since these manipulations bias intake of tasty foods even when they are non-calorific.

*SYNTHESIS: THE NUCLEUS ACCUMBENS CONTRIBUTES TO "WANTING", "LIKING" AND LEARNING*

Different neurotransmitters have different actions within the nucleus accumbens. Dopamine seems to contribute to learning and the impact of conditioned stimuli on behaviour, whereas GABA and glutamate *additionally* mediate 'primary' motivating behaviours such as feeding and hedonic attribution. Additionally, nucleus accumbens neurons in different anatomical locations ('ensembles' (Pennartz et al. 1994)) seem to contribute differentially to the effects of 'primary' motivating stimuli (motivation to gain reward or avoid punishment and attachment of hedonic value to stimuli are all implicated in the shell) versus the effects of 'secondary' motivating stimuli (although associative learning is implicated shell, the behavioural effects caused by conditioned stimuli, including actions made towards incentive stimuli that are reinforced, e.g. bar-pressing for reward, are all implicated in the core).

## CORRELATIONS BETWEEN BEHAVIOUR AND MESOACCUMBENS NEURAL ACTIVITY

### SINGLE-NEURON RECORDINGS FROM MIDBRAIN DOPAMINE NEURONS

#### *CHARACTERISATION OF DOPAMINE NEURONS*

Directly identified dopamine neurons have been recorded intracellularly in the in the substantia nigra pars compacta of the anaesthetised rat (Grace and Bunney 1983, 1980). The waveform characteristics and firing patterns of these neurons were similar to those obtained from extracellular recordings of putative midbrain dopamine neurons of the anaesthetised (Bunney et al. 1973; Wang 1981) and freely moving rat (Freeman et al. 1985). Dopamine neurons were identified as having long duration action potentials (>2ms) with a biphasic or triphasic shape and a low 'spontaneous' firing rate (range 1-10Hz) that could switch to bursts of spikes. Additionally, administration of dopamine agonists and antagonists inhibited and excited, respectively, the firing rates of dopamine neurons, possibly by activation of autoreceptors on dopamine neurons (Bunney et al. 1987).

However, mesocortical dopamine neurons have low numbers of autoreceptors and are unaffected by administration of dopamine agonists (Chiodo et al. 1984). Furthermore, some neurons in the ventral tegmental area that do not release dopamine have dopamine receptors and can be inhibited by dopamine agonists (Kiyatkin and Rebec 1998). Thus, administration of dopamine agonists/antagonists seems an inaccurate way to identify dopamine neurons. Baseline firing rate also seems to be a problematic measure since dopamine neurons projecting to the prefrontal cortex have a much higher average firing rate (mean 9Hz) and a greater degree of bursting compared to dopamine neurons that project to the striatum (Chiodo et al. 1984). Finally, the criterion that dopamine neurons have action potential duration of >2ms is not completely accurate since the more predictive characteristic of dopamine *versus* non-dopamine neurons in the anaesthetised rat was that the duration from action potential onset to the negative trough >=1.1ms (Ungless et al. 2004). Thus,

recording the activity of single midbrain dopamine neurons in behaving animals remains a problematical issue. Current criteria that dopamine neurons have a firing rate <10Hz, can fire in bursts, and the duration from action potential onset to the negative trough >=1.1ms are useful to confirm the neuron is dopaminergic. However, many dopamine neurons (e.g. those that project to the cortex) are likely to be missed. Perhaps the only way to avoid misclassification would be to report the data from all neurons recorded in a midbrain area, as has been done recently in the ventral tegmental area of the rat (Kiyatkin and Rebec 1998, 2001).

*PHASIC RESPONSES OF DOPAMINE NEURONS*

Dopamine neurons in areas A8, A9 and A10 of the *Macaca fascicularis* (macaque) monkey, identified on the basis of the electrophysiological characteristics described by Freeman et al. (1985), respond to motivationally arousing stimuli that determine behavioural reactions (Ljungberg et al. 1992). Importantly, these responses are context-dependent and adapt over repeated presentations. Thus, it has been demonstrated that dopamine neurons respond with a stereotyped, phasic excitatory burst (onset latency <100ms, burst duration <200ms) to appetitive events such as the unexpected delivery of reward outside of a task (Mirenowicz and Schultz 1994) and to reward delivery during the early learning stages of various reinforcement tasks (Schultz et al. 1993). Importantly, during task learning responses seem to shift from reward delivery to reward-predictive cues (Hollerman and Schultz 1998; Ljungberg et al. 1992; Mirenowicz and Schultz 1994; Romo and Schultz 1990; Schultz et al. 1993). Indeed, during the learning of successive tasks, responses can transfer between reward-predictive cues (Schultz et al. 1993). It has recently been demonstrated that phasic bursts of activity to reward delivery encodes information on the relative value of reward compared to that predicted and responses to reward-predictive stimuli can signal the probability and relative magnitude of an upcoming predicted reward (Fiorillo et al. 2003; Tobler et al. 2005).

Dopamine neurons can also respond with pure phasic inhibitions at a slightly later latency than the excitations (~100-400ms (Hollerman and Schultz 1998)). Inhibitions occur at apparently 'negative' events such as the presentation of aversive or aversive-predictive stimuli (Mirenowicz and Schultz 1996), stimuli that predict the absence of reward (Tobler et al. 2003), absence of predicted reward delivery (Hollerman and Schultz 1998; Ljungberg et al. 1991; Schultz et al. 1993; Waelti et al. 2001) and delivery of a reward with a lower relative value to that predicted (Tobler et al. 2005). A third type of dopamine response reported is a biphasic excitation-inhibition to novel or non-rewarding salient stimuli that trigger orienting reactions (Horvitz et al. 1997; Schultz 1998; Steinfels et al. 1983; Strecker and Jacobs 1985). These responses disappear at the time when the orienting reaction habituates (Steinfels et al. 1983). Biphasic responses have also been reported at presentation of neutral stimuli that are physically similar to reward-predictive stimuli (Schultz 1998; Waelti et al. 2001) and to compound stimuli comprised of a stimulus predictive of reward and a stimulus predictive of the absence of reward (Tobler et al. 2003).

*DO PHASIC DOPAMINE RESPONSES SIGNAL A REWARD-PREDICTION ERROR?*

Schultz and colleagues have provided a theoretical explanation attempting to encapture the data described above. It has been proposed that dopamine neurons receive separate sets of information regarding the predicted value of incentive stimuli (reward or reward-predictive stimuli) for successive time points in the future, and the time and relative value of incentive stimuli when they occur. The phasic output activity of dopamine neurons encodes the discrepancy between these two sets of information (Montague et al. 1996; Schultz 1998; Schultz et al. 1997). Thus, excitatory responses arise when the occurrence of a stimulus is better than that predicted (e.g. unexpected reward delivery), inhibitions signal the delivery (or absence) of a stimulus that is worse than predicted (e.g. reward omission), and there is no response when a stimulus occurs that was fully predicted (e.g. expected reward delivery). Together, these phasic responses are hypothesised to signal a 'reward-prediction error'. During

learning, as incentive stimuli become predicted, the response 'transfers' to the earliest unpredicted, reward-predictive stimuli. Additionally, Schultz (1998) has proposed that the phasic dopamine response acts as a 'teaching signal' to update future reward-prediction information and modify current behavioural selection.

However, there are two possible inconsistencies with this theory, which suggest phasic dopamine responses might code an error in prediction of *any salient stimulus* rather than in prediction of *reward,* per se (Horvitz 2000; Redgrave et al. 1999b). First, dopamine neurons respond to novel or salient stimuli that are not reward-related. Although it is possible that these stimuli are *potentially* rewarding (Schultz 1998), they could also be potentially aversive (Redgrave et al. 1999b). Second, a differential number of dopamine neurons respond to aversive-predictive (~30%) *versus* reward-predictive (~78%) stimuli (Mirenowicz and Schultz 1996). If dopamine neurons respond to aversive stimuli to signal the occurrence of a stimulus with a lower-than-predicted reward value then one would expect equal numbers of depressions to aversive-predictive stimuli as excitations to reward-predictive stimuli. Alternatively, if dopamine neurons only process stimuli directly related to rewarding, not aversive, events then there would be no responses expected to aversive-predictive stimuli. However, the differential numbers of responses to aversive *versus* rewarding stimuli suggest that responses might have reflected differential stimulus salience. Consistent with this, microdialysis and voltammetry experiments (described below) have found changes in extracellular dopamine to strongly aversive or aversive-predictive stimuli but not to mild aversive stimuli akin to those used by Mirenowicz and Schultz (1996) (Horvitz 2000; Salamone 1994).

*COULD DOPAMINE RESPONSES ENCODE SWITCHING RATHER THAN REWARD-PREDICTION ERROR?*

Redgrave and colleagues have proposed that dopamine responses might be used in the process of switching of attentional and behavioural resources to any

salient stimulus and not to code reward-prediction error (Redgrave et al. 1999a, b). Depending on the efferent connections of dopamine neurons, responses could trigger switching between the selection of goal strategies ('limbic' striatum), individual actions within a goal ('cognitive' striatum) or individual muscles within an action ('motor' striatum). Indeed, in the studies carried out by Schultz et al., phasic dopamine responses typically correlate with presentation of rewards or reward-predictive stimuli *and* with the monkey switching an aspect of its behaviour (e.g. moving from rest to lever press, reaching to consume reward, moving eyes to a spatially located picture).

In one of these experiments, the switching hypothesis might provide a more parsimonious explanation of the data than the reward-prediction error hypothesis (Schultz et al. 1993). Monkeys were trained to press one of two levers to earn reward following presentation of a trigger cue. The trigger cue was preceded 1s earlier by the presentation of an instruction cue, which signalled the lever that if pressed would bring reward. During learning of this task, the phasic dopamine response transferred from reward delivery to the presentation of the instruction cue. However, when a 2.5-3.5s variable delay was introduced between instruction and trigger cue, excitatory responses were found at both cue presentations. The reward-prediction error model seems to account for these data since delay of a predicted upcoming incentive stimulus makes the current 'predicting' incentive stimulus of relatively lower value (Montague et al. 1996). Indeed, in the above example, the introduction of delay caused dopamine responses to move forward in time to the trigger stimulus. However, the model would also predict *less* excitation to the instruction cue since the introduction of a longer delay would make the instruction cue predictive of a relatively lower valued reward. However, these responses remained at the same level. Interestingly, the switching hypothesis might provide a more parsimonious account of these data since the presence of saccades (behavioural switches) to the instruction and/or trigger cues corresponded with the presence of dopamine responses. Indeed, it has recently been reported that monkey dopamine

responses might trigger switches between learned sets of behaviours within a one-direction memory guided saccade task (Takikawa et al. 2004). However, to date, no single-neuron recording study has teased apart reward-related *versus* behavioural switching processes.

*SELF-ADMININSTRATION*

Different lab groups have recorded the activity of single nucleus accumbens neurons in rats during self-administration (one bar-press required to gain reward) of cocaine, heroin, food and water rewards. These studies report phasic (a few seconds) and tonic (minutes) changes in activity prior to and following the bar press. The most common activity reported is excitation *prior* to the bar press (Carelli and Deadwyler 1994; Chang et al. 1998; Peoples and West 1996), phasic excitation or inhibition *after* the bar press (Carelli and Deadwyler 1994) and *tonic* inhibition in firing after the bar press (Chang et al. 1998; Peoples and West 1996). Single neurons tended to respond similarly to self-administration of food and water rewards, but differently to cocaine *versus* food or water (Carelli et al. 2000) or cocaine *versus* heroin rewards (Chang et al. 1998). These data suggest that neurons respond similarly during self-administration of natural reinforcers and differently to different drug reinforcers.

Importantly, these responses were attenuated when bar-presses were no longer followed by reinforcement, suggesting that increases in activity prior to bar pressing reflects anticipation or expectation of reinforcement rather than response preparation, *per se* (Hollander et al. 2002). Greater magnitudes in anticipatory responses have been reported in the core *versus* medial shell of the nucleus accumbens (Ghitza et al. 2004). Tonic decreases in activity were not tightly correlated with general locomotion (Peoples et al. 1998) or to individual bar presses (Nicola and Deadwyler 2000). In some neurons, post-press changes could reflect sensory aspects of the reinforcer and/or associated stimulus since responses remained when cocaine and the conditioned stimulus was delivered non-contingently (Peoples et al. 1997). Other post-press responses might be related to the action-outcome contingencies since patterns of activity can disappear during non-contingent presentations of cocaine (Peoples et al. 1997) and can remain following unreinforced bar presses (Ghitza et al. 2004).

*GOAL-PREDICTING RESPONSES*

In more complex tasks it has been found that the activity of striatal neurons in the monkey is correlated with the preparation, execution or withholding of movements. However, it is unlikely that these responses directly trigger movements, since they were typically modulated differentially between trials where the monkey worked for an upcoming reward *versus* conditioned reinforcer (Hollerman et al. 1998). Similarly, bar release responses (required to gain reward) were attenuated when the animal was no longer cued as to the proximity of reward (Shidara et al. 1998). Moreover, responses to reward-predictive cues in this schedule were not linearly related to reaction times (Bowman et al. 1996). It has also been reported that neurons that responded differentially to cues predictive of go and no-go behaviours lost, rather than reversed, responsiveness when go and no-go responses to the cues were reversed by changing the motivational significance of cues (Setlow et al. 2003).

It has been found that neural responses to reward-predictive cues can be stronger when a subsequent behavioural response is made (Nicola et al. 2004b). These excitations were typically followed by inhibitions during reward consumption and Nicola et al. suggested that these responses might encode switching from appetitive to consummatory behaviours. However, it is possible that differential responses reflected differential predictions of reward on trials where a behavioural response was made *versus* not, rather than signals to trigger the behavioural response. In summary, it seems that activity of single nucleus accumbens neurons predicts upcoming reward and this neural information could then be used to bias the subsequent behavioural choice without triggering individual behaviours.

There is also evidence that nucleus accumbens responses are involved in learning the motivational significance of cues and cue-response associations. Thus, primate striatal neurons adapted their response with behavioural reactions

during learning of new predictive cues and sometimes the neural changes preceded the behavioural changes (Tremblay et al. 1998). Furthermore, Setlow et al. (2003) demonstrated that neurons that had responded differentially to reward-predictive *versus* aversive-predictive cues, independently of the subsequent 'go' versus 'no-go' behavioural response, reversed their response when the motivational significance of the cues was reversed.

It has been argued that cue-responses in the nucleus accumbens are not reward-*predictive*, such as in the manner described by Schultz et al. for dopamine neurons (Schultz 1998), but schedule-informative (Shidara et al. 1998). This has been suggested because neurons responded in a variety of ways to some cues that signalled different proximities and amounts of work required to gain reward but not others and the pattern did not seem to reflect a transfer of response to the earliest predictor of reward. However, it remains a possibility that responses were transferred to the earliest predictor of reward, but different neurons had different learning rates, resulting in the complex pattern of responses found by Shidara et al. (1998). Consistent with this, nucleus accumbens neural responses to reward-predictive stimuli disappeared when the ventral tegmental area was temporarily inactivated or following microinjections of dopamine antagonists into the nucleus accumbens (Yun et al. 2004b). Additionally, cue responses do not simply encode the upcoming presence of reward but the upcoming type (Hassani et al. 2001) and magnitude (Cromwell and Schultz 2003) of reward, akin to a recent report that dopamine neurons predict the upcoming reward value (Tobler et al. 2005). Since nucleus accumbens neurons can respond to aversive-predictive stimuli (Roitman et al. 2005; Setlow et al. 2003; Williams et al. 1993; Yanagimoto and Maeda 2003) it seems that their responses contain goal-predictive information with subsets of neurons encoding different predictions for different goals.

*REWARD ACTIVITY*

As described earlier neurons respond phasically and tonically after the bar press during self-administration of rewards. Additionally, it has been found that neurons

in the monkey striatum can respond to a drop of juice of reward with excitations similar to those found to reward-predictive stimuli (Bowman et al. 1996; Cromwell and Schultz 2003; Hassani et al. 2001; Shidara et al. 1998). However, in these studies phasic changes to reward might reflect responses to reward as a reward-predictive stimulus, based on the prediction from the preceding action. In other words, reward detection might be functionally equivalent to a reward-predictive response since detection of rewards predicts upcoming pleasure. In line with this argument, it has been reported that neurons responding to conditioned stimuli are the same neurons as those that respond following the reinforced bar press, and they do so with the same valence (Carelli and Ijames 2001). Moreover, pre- and post-bar press responses disappeared when the reward and conditioned stimulus were omitted (Carelli and Ijames 2000; Hollander et al. 2002) or when cocaine reward was delivered unpredictably in the absence of predictive stimuli (Carelli 2002). These 'reward detection' responses might be additional to consummatory responses since brief excitations at the time of cue offset/reward onset can then be followed by excitation and inhibition over longer time periods during consumption (Roitman et al. 2005). Thus, it seems that neurons respond to reward detection and these are probably functionally equivalent to reward-predictive responses.

However, there are neural changes during reward delivery that are different to reward-predictive responses. For instance, tonic changes in firing (typically inhibitions) have been identified during and following heroin and cocaine administration that can last for minutes after the drug infusion and is often opposite in valence to the activity preceding reward (Chang et al. 1998; Peoples and West 1996). Recently, Nicola et al. (2004) assessed reward consumption in the rat where the operant response to a reward-predictive stimulus was separated from reward. They found activity after entry to the reward receptacle that lasted several seconds, presumably during consumption of reward. This activity was predominately inhibitory and was present even when reward was not delivered.

It has also been recently reported that some nucleus accumbens neural responses (usually inhibitory) were present during reinforced and unreinforced lick bouts and were implicated in mediating the onset and maintenance of consummatory behaviours. However, other responses (usually excitatory) occurred only when reward was delivered, began after the onset of the lick bout and showed differential activity depending on the sensory aspects of reward or its relative palatability (Taha and Fields 2005). These neurons were implicated in mediating taste palatability. There is also a recent report that neurons responded innately (prior to any previous experience) to rewarding sucrose and aversive quinine, typically with inhibition and excitatory responses, respectively. These responses were also related to oral-motor behaviour (Roitman et al. 2005). In both these experiments there were no differences in the type of responses found between core *versus* shell neurons. Again these results suggest that responses could drive consummatory behaviour and/or reflect taste. Together with the data of Taha and Fields (2005) it does not seem that excitations necessarily encode palatability since quinine responses were often excitatory. Future work testing responses to rewarding and aversive stimuli of differing values and with more detailed analysis of anatomical location (e.g. rostrocaudally) might provide more answers as to the contribution of these responses to consummatory behaviours, detection of salient primary stimuli and hedonic attribution.

*SYNTHESIS OF SINGLE-NEURON RECORDINGS IN THE MESOACCUMBENS DOPAMINE SYSTEM*

Dopamine neurons primarily respond to reward-predictive stimuli and unpredicted reward delivery. These responses might encode a discrepancy between the value and time of the reward predicted and the value and time of the reward received. It is possible that these responses are used in the striatum to learn reward-predictive stimuli. Alternatively, learning might be processed by different neurons that project to dopamine neurons and dopamine responses could instead facilitate attentional and/or behavioural switching. Nucleus accumbens neurons also respond to goal-predictive stimuli, responses that can adapt

throughout learning and often bias behavioural selection. Similarly, there are also responses immediately at the onset of reward delivery, which might encode reward detection (akin to dopamine neurons). These responses seem functionally equivalent to the responses found at reward-predictive stimuli, although it is often difficult to dissociate reward detection responses from reward-predictive responses. However, there have been recent reports that nucleus accumbens neurons respond throughout reward delivery in a manner that might contribute to the initiation of consummatory behaviours and/or hedonic attribution of unconditioned stimuli.

*MICRODIALYSIS*

Burst firing of dopamine neurons causes a phasic release of dopamine from the pre-synaptic terminal that diffuses to neurons beyond the post-synaptic terminal. This 'extracellular' dopamine level is continually in a flux between burst firing releasing dopamine *versus* reuptake of dopamine back into the neuron (Wightman and Robinson 2002). Measurements of extracellular dopamine levels using microdialysis techniques have demonstrated a differential involvement of dopamine in the nucleus accumbens core, shell and prefrontal cortex, evidence that cannot currently be provided by neurophysiological techniques (Di Chiara 2002). In the shell, extracellular dopamine is increased during consumption of palatable rewards (chocolate, sucrose), which habituates on a subsequent presentation. Shell dopamine is phasically reduced to aversive stimuli such as tail pinch, quinine administration, or saccharin administration after it was paired with an aversive stimulus (dopamine was increased to saccharin prior to the pairing). Additionally, shell dopamine is increased by aversive-predictive stimuli but not reward-predictive stimuli. In contrast, dopamine in the core is enhanced to both aversive and rewarding stimuli and non-contingent reward-predictive stimuli, but not to aversive-predictive stimuli (Bassareo et al. 2002; Di Chiara 2002).

Di Chiara (2002) argues that administration of addictive drugs cause a preferential increase in extracellular dopamine in the nucleus accumbens *versus* dorsal striatum and a selective (non-psychostimulant drugs, e.g. heroin, ethanol) or preferential (psychostimulant drugs, e.g. cocaine, amphetamine) increase in dopamine in the shell *versus* core of the nucleus accumbens. Importantly, dopamine increase to drug administrations does not habituate with subsequent exposure. Di Chiara theorises that enhanced dopamine in the shell, which typically occurs during delivery of unpredicted rewards, and drugs of abuse, stimulates associative learning of the reward-predictive stimuli. Since this response does not habituate during drug delivery, very strong associations with

drug-predictive stimuli are formed. In contrast dopamine released in the core, which occurs to appetitive and aversive motivational stimuli and does not habituate, is proposed to contribute to the expression of motivated behaviour. Addictive drugs sensitise the expression of this motivated behaviour since the predictive stimuli have become such strong incentives. This associative learning explanation of the function of dopamine accounts for drug addicts having abnormal sensitivity to *drug*-associated stimuli rather than to any motivationally salient stimulus as proposed by Berridge and Robinson (1998) (e.g. *reward* "wanting").

*ELECTROCHEMISTRY*

Electrochemical techniques typically oxidise molecules within the brain, which can change a measured electrical current. Two main electrochemical techniques have been used to measure extracellular dopamine in rats: chronoamperometry and fast-scan cyclic voltammetry. With the former technique it has typically been found that dopamine levels increase during reward seeking (e.g. heroin, food, cocaine reinforcement) and decrease during reward taking, and these changes are correlated with biphasic changes in movement (Kiyatkin 2002). Although these findings are in agreement with the tonic activity of ventral tegmental area neurons that are presumed to be dopaminergic (Kiyatkin and Rebec 2001), the results are at odds with microdialysis studies (no correlation between decreases in dopamine and reward delivery) and are difficult to explain by the known dopamine-enhancing actions of drugs such as cocaine. More importantly, the technique has been called into question since the 'dopamine' signal includes other molecules that appear like dopamine (Carelli and Wightman 2004; Di Chiara 2002; Wightman and Robinson 2002).

However, extracellular dopamine has also been measured using fast-scan cyclic voltammetry and this technique has superior isolation of dopamine molecules, a finer temporal resolution (100ms) and produces results that are consistent with microdialysis studies, the known actions of drugs, and the neurophysiological

work by Wolfram Schultz. It was found using this technique that extracellular dopamine throughout the striatum increased phasically to the introduction to male rats of conspecifics (Robinson et al. 2002). Within the nucleus accumbens the highest number of dopamine increases was found during the introduction of a receptive female *versus* male conspecific, and this effect habituated on subsequent presentation. These activations continued throughout copulation (although at a reduced level) and were rarely found at the consummatory stages of copulation, suggestive of a role in reward-seeking, not reward-taking behaviour. Similarly, dopamine levels in the nucleus accumbens core area increased following the presentation of a food-predictive cue and peaked at the bar press made to deliver food (Roitman et al. 2004). Moreover, an attenuated signal was found following presentation of food-predictive cues that were not followed by a bar press and dopamine was released later in the trial prior to the bar press in the absence of the cue. The authors suggest that enhanced dopamine might facilitate reward-seeking since comparable results were found within a similar task during self-administration of cocaine (Phillips et al. 2003). These findings are consistent with the hypothesis that dopamine release in the nucleus accumbens core to conditioned stimuli might mediate the expression of motivated behaviour (Di Chiara 2002).

*SUMMARY*

Behavioural correlates of extracellular dopamine from fast-scan cyclic voltammetry and microdialysis techniques are largely in agreement with the neurophysiological findings of Schultz et al. However, the work reviewed by Di Chiara has added some important information. First, changes in extracellular dopamine vary between terminal regions (core, shell and prefrontal cortex). Second, aversive stimuli cause *increases* in extracellular dopamine in the core and *decreases* in the shell, data that are largely unaccounted for by Schultz. Third, it is possible that different dopamine neurons respond to unpredicted reward than conditioned stimuli since Di Chiara implicates dopamine increases in the shell to the former and dopamine increases in the core to the latter. If this

were the case then the dopamine response might not transfer within neurons as has been previously suggested (Schultz 1998). Lastly, Di Chiara proposes that dopamine in the shell mediates associative learning between stimuli and their physiological outcome (and indirectly in instrumental learning) whereby dopamine is released differentially in relation to motivational valence, whereas dopamine release in the core mediates response expression to motivationally *salient* stimuli. However, Schultz and colleagues have not reported different dopamine responses correlating with learning *versus* response expression. It is possible that these differences (or at least some of them) are due to tonic dopamine responses that have not been investigated by Schultz et al.

*ACTIVITY CORRELATED WITH REWARD-PREDICTION ERROR*

Through the use of event-related functional magnetic resonance imaging (fMRI) changes in blood flow can be measured throughout the whole of the human brain and the activity in identified regions of interest can be correlated with task-related events and behavioural responses. It has been suggested that the blood-oxygen-level-dependent (BOLD) signal from fMRI is most likely to correspond to inputs and local processing within a given area rather than its output spiking activity (Logothetis et al. 2001). It has been found that changes in BOLD activity in the striatum can correlate with the temporal reward prediction error (McClure et al. 2003; Pagnoni et al. 2002), that has previously been implicated in the burst activity of dopamine neurons (Schultz 1998). In the former study, passive subjects received conditioned stimulus-juice reward associations. In catch trials when expected reward was delayed, a negative prediction error occurred at the time of unexpected omission of reward and a positive prediction error when the delayed juice was unexpected delivered. Correspondingly, there was a reduction and increase in putamen BOLD activity, respectively (McClure et al. 2003). In a similar task where subjects had to press a button following a discriminative stimulus signalling the availability of juice reward there was an increase in BOLD within the nucleus accumbens, although this was at the time of unexpected omission of reward, which produces a negative prediction error (Pagnoni et al. 2002). Many other studies implicate nucleus accumbens BOLD activation in reward processing, which although not directly tested, could reflect errors in reward prediction. For example, activity has been correlated with the prospect and receipt of monetary reward (Breiter et al. 2001; Elliott et al. 2003), self-reported cocaine craving (Breiter et al. 1997), viewing attractive faces where the eyes are directed at the subject (Kampe et al. 2001) and reading of humourous cartoons (Mobbs et al. 2003).

This work has been extended to show that activity correlated with reward prediction error is present in the ventral striatum during instrumental conditioning and classical conditioning (O'Doherty et al. 2004). In one task subjects had to choose to respond to one of two stimuli (one stimulus was more reward-predictive than the other) in an attempt to gain juice reward (instrumental conditioning) whereas in another task the computer chose the stimulus and the subject had to respond to it (classical conditioning). Reward-prediction error activity was present in the ventral striatum in both tasks, although was only present in the dorsal striatum during instrumental conditioning. The authors argue that these data are consistent with the hypothesis that a reward-prediction error signal from dopamine neurons is processed differentially between the ventral and dorsal striatum. Ventral striatal neurons interact with dopamine neurons to identify reward-predicting stimuli, possibly through plasticity effects on cortical inputs, whereas dorsal striatal neurons use the reward-prediction error signal to learn stimulus-response or stimulus-response-reward associations. It should be noted that this actor-critic model of reinforcement has been used previously to explain dopamine-striatal interactions in this manner (Schultz 1998).

*ACTIVITY CORRELATED WITH SALIENT OR AVERSIVE STIMULI*

However, as has been argued previously, dopamine-striatal interactions may not merely process reward-predicting stimuli (Horvitz 2002; Redgrave et al. 1999b; Salamone et al. 1997). Indeed, it has been found using fMRI that activity can be increased in the striatum by non-rewarding salient stimuli and aversive stimuli. For instance, it has been demonstrated that BOLD activity in the nucleus accumbens was increased to infrequent (more salient) *versus* frequent (less salient) presentations of distracting stimuli whilst subjects were performing a simple target discrimination task (Zink et al. 2003). This suggests the nucleus accumbens can process salient, non-rewarding stimuli. In a separate task it was found that there was increased activation in the nucleus accumbens *and* caudate to infrequent *versus* frequent distracting stimuli when the subject was required to respond to stimuli presentation. These data mirror the actor-critic interpretation of

ventral-dorsal striatal processing made by O'Docherty et al. (2004) except activity changes were in relation to unpredicted *salient* stimuli rather than unpredicted *reward* stimuli, *per se*. Indeed, Zink et al. (2003) suggested that the nucleus accumbens might have been recruited to perform attentional switches to the distracting stimuli and the caudate, behavioural switches. Similarly, it has been found that there was increased BOLD activity in the ventral striatum when subjects had to make an attentional and behavioural switch by responding to one of two stimuli on the basis of a simultaneously presented rule cue, yet activations did not occur when the rule cue was switched (Cools et al. 2004).

Moreover, BOLD activation in the nucleus accumbens/ventral striatum has been reported to delivery of painful thermal stimuli (Becerra et al. 2001) and to anticipation of 'unpleasant' electric stimulation of the finger (Jensen et al. 2003). In this latter study it was demonstrated that there was increased activity in the nucleus accumbens following the onset of a stimulus that predicted an electric shock to the finger, both when subjects could not control administration of the shock (passive condition), and when they could respond to the cue to avoid the shock (active condition). The authors suggested a role for nucleus accumbens processing in motivational salience as has previously been postulated by Berridge and Robinson (1998). Although delivery of low valued reward (Elliott et al. 2003) and anticipation of monetary losses (Knutson et al. 2001) have failed to demonstrate changes in the nucleus accumbens, Jensen et al. (2003) argue that salient aversive stimuli, such as those inducing pain, are required to activate the nucleus accumbens.

Counter to this, it has been demonstrated, both in the activity of dopamine neurons and in fMRI signals, that activity is *decreased* during omission of reward and *increased* during unexpected reward delivery, yet both stimuli are arousing and salient (McClure et al. 2003; Schultz 1998). Therefore, it seems that akin to previous neurophysiological and microdialysis work fMRI studies have found evidence that the nucleus accumbens processes information on the motivational

valence and predictability of stimuli and/or the motivational salience of stimuli that can trigger attentional and/or behavioural switches. From previous studies demonstrating that aversive and rewarding stimuli may be processed differentially between core and shell (Di Chiara 2002), or even within different regions within the shell (Reynolds and Berridge 2003, 2002), it is possible that different neurons encode motivational valence *versus* motivational salience as proposed by Di Chiara (2002). Unfortunately, current fMRI techniques do not have the spatial resolution to detect these putative differences across different anatomical locations in the nucleus accumbens.

## RESEARCH QUESTIONS

From the above review it seems clear that the mesoaccumbens dopamine system is implicated in processing motivationally salient stimuli and the behavioural reactions to these stimuli. However, the precise operational mechanism of these processes is unclear. It is possible that nucleus accumbens and dopamine neurons compute reward prediction. Alternatively, nucleus accumbens neurons could respond equivalently to rewarding and aversive stimuli to encode motivational salience, acting as a filter to react to environmentally important stimuli. Are aversive and reward stimuli within an operant task processed by individual nucleus accumbens in a manner that suggests motivational valence or motivational salience? What behaviours might nucleus accumbens facilitate? They could bias selection of particular responses, a selection of responses or facilitate switching between behavioural strategies. Similarly, do dopamine neurons encode unpredicted stimuli in relation to reward in order to "teach" reinforcement learning, or in relation to salience to switch attention and/or behaviour? Nucleus accumbens neurons have been implicated in goal-prediction, learning, goal "wanting", goal detection, goal consummatory behaviours and goal "liking". If neurons are involved in learning reward-predictive stimuli, do they do so in a manner similar to dopamine neurons or do they provide schedule information? Since different neurotransmitters can have differential effects on feeding *versus* reward-seeking, are these processed differentially by individual neurons or similarly by different neurons? Furthermore, do individual neurons respond to reward in the same manner as they do to reward-predictive stimuli? Finally, do nucleus accumbens neurons that respond to reward delivery encode reward "liking", can these neurons also encode reward "wanting", and if so do they respond to "wanted" and "liked" stimuli in the same manner?

We sought to answer at least some of these questions by recording the activity of single nucleus accumbens and dopamine neurons in the rat within a variety of

behavioural tasks. In the first experiment, we aimed to record the activity of nucleus accumbens neurons during a second-order schedule of saccharin reinforcement. In this task intermittent presentations of conditioned stimuli reinforce long chains of behaviour that ultimately deliver reward (Everitt and Robbins 2000). This experiment would allow us to assess how single neurons respond to conditioned stimuli that are reinforcers of behaviour and are temporally separated from reward. Moreover, we would be able to compare neural responses to conditioned reinforcers *versus* reward (primary reinforcers). We might also find information on whether neural responses to conditioned stimuli bias the selection of specific behaviours or a longer chain of behaviour. Additionally, since conditioned stimuli occur at different temporal proximities to reward, we might be able to assess whether neurons are biased in responding to the earliest reward predictor only (as dopamine neurons might be expected to), or to conditioned stimuli at different points within the schedule, as if to provide the animal with schedule placement information. We also wanted to discover whether nucleus accumbens and dopamine neurons encode behavioural switching or motivational valence information, and set out to do so by developing a behavioural task designed to explicitly test these hypotheses. Finally, we wanted to develop a behavioural task that we could use for future neurophysiological testing to test the effects of reward "wanting" and "liking" on neural responses in individual neurons.

## CHAPTER 2

# SECOND-ORDER STIMULI DO NOT ALWAYS INCREASE OVERALL RESPONSE RATES IN SECOND-ORDER SCHEDULES OF REINFORCEMENT IN THE RAT

The work presented in this chapter has been published previously
(Wilson and Bowman, 2004, *Psychopharmacology (Berl)*, 174: 430-437).

**ABSTRACT**

Second-order schedules of reinforcement have been used extensively to model reward-seeking (Everitt et al. 1987) and drug-seeking behaviour (Schindler et al. 2002). Second-order stimuli within second-order schedules have been shown to enhance response rates during operant responding for natural reinforcers (Everitt et al. 1989) and drug reinforcers (Arroyo et al. 1998; Goldberg et al. 1979). This has led some to view second-order schedules of drug reinforcement as a model of drug-seeking in addicts maintained by drug-associated stimuli (Pilla et al. 1999; Spealman et al. 1999). However, the functional role of the second-order stimulus within second-order schedules is complex. We investigated the role of second-order stimuli within a second-order schedule of reinforcement (FI 4min (FR10: S)) maintained by sweetened water reinforcement. Eight rats were trained to press a bar on a second-order schedule of reinforcement and tested in the presence and absence of the second-order stimulus. In contrast to most previous work overall bar-pressing rates were significantly increased when the second-order stimulus was omitted (second-order stimulus omission: 0.17Hz (± 0.04 95% C.I.); second-order stimulus present: 0.13Hz (± 0.04 95% C.I.)). However, second-order stimuli also changed the pattern of responding whereby rats would make a bout of bar presses prior to the presentation of the second-order stimulus and then pause briefly after the second-order stimulus. In the absence of second-order stimuli, responding was uniformly high. Control measures, such as the second-order stimulus' ability to evoke checking for the primary reinforcers, indicated that the second-order stimulus was associated with the primary reinforcer. These results demonstrated that although second-order stimuli maintained responding and caused the rat to check for primary reinforcement, overall response rates were increased when the second-order stimuli were omitted. This has implications for interpreting the results of studies where overall response rates within second-order schedules have been the only measure used to assess the effects of potential anti-addiction drugs. Future studies could be improved by performing a second-order stimulus omission test analysing both the

overall response rates and the temporal organization of responding with respect to the second-order stimulus.

## INTRODUCTION

Second-order schedules of reinforcement were developed to enable the study of conditioned reinforcement for extended periods of time while avoiding extinction (Kelleher 1966). Recently they have been used on rats to model reward-seeking behaviour. Responding during second-order schedules is said to reflect the reinforcing efficacy of drug or natural rewards and associated stimuli, whilst removing some of the confounding effects on responding that are a result of receiving the reward, such as the psychomotor effects of amphetamine (Everitt and Robbins 2000). Responding in second-order schedules is reinforced not solely by primary reinforcement, since it is often enhanced by the intermittent presentations of a second-order stimulus. When the second-order stimulus is omitted from the second-order schedule (often referred to as conditioned stimulus/reinforcer omission), global levels of responding are significantly decreased, and when reinstated global levels of responding return to baseline levels (Alderson et al. 2000b; Arroyo et al. 1998; Goldberg et al. 1979; Goldberg and Tang 1977; Katz 1979; Kelleher 1966; Parkinson et al. 2001).

Many studies have attempted to discover the brain processes active during second-order schedules (Alderson et al. 2000a; Everitt et al. 1989; Hutcheson et al. 2001; Ito et al. 2000; Kantak et al. 2002a, b; Weissenborn et al. 1997; Whitelaw et al. 1996). Other studies have used second-order schedules to model drug craving and relapse (Di Ciano and Everitt 2002; Pilla et al. 1999). In this regard, second-order schedules have been used extensively to screen drugs that reduce drug-seeking behaviour in animals (Backstrom and Hyytia 2003; Cervo et al. 2003; Czoty et al. 2002; Di Ciano and Everitt 2001, 2003b; Di Ciano et al. 2003; Kantak et al. 2001; Kantak et al. 2000; Khroyan et al. 2000; Mello and Negus 2001; Negus and Mello 2003, 2002; Park et al. 2002; Pilla et al. 1999; Platt et al. 2003; Platt et al. 2001).

A variety of reinforcers have been used to maintain second-order schedules including food, sex, cocaine, heroin, d-amphetamine, morphine, nicotine, ethanol, $\Delta^9$-tetrahydrocannabinol and phencyclidine (Schindler et al. 2002). Although sweetened fluid (strawberry-raspberry flavoured glucose solution) has recently been used to maintain a second-order schedule in rhesus monkeys, there was no testing performed under second-order stimulus omission (Comer et al. 2002; Evans et al. 2003). In preparation for future neurophysiological studies, we used sodium saccharin solution (sweetened fluid) as the primary reinforcer, since sodium saccharin has no calorific content and does not produce metabolic changes in the brain (Messier and White 1984; White and Carr 1985). We hypothesized that this primary reinforcer would maintain responding under a second-order schedule and that responding would be additionally maintained and enhanced by the second-order stimulus. Therefore, we predicted that omission of the second-order stimulus from the second-order schedule would decrease responding, and reinstatement of the second-order stimulus would restore responding back to baseline levels. In contrast to our predictions, the presence of the second-order stimuli inhibited overall response rates. We have submitted these findings because we believe that this highlights the need for more detailed routine analysis of responding during second-order schedules of reinforcement, specifically as it relates to animal models of drug-taking.

## METHODS

### SUBJECTS

Eight Lister Hooded adult male rats (Harlan UK), weighing 378-487g when training began, were housed in quadruplets on a light 12h: dark 12h light cycle. The rats were on a regime of restricted water access during behavioural training and testing. During training free access to water was provided from 4-5PM each weekday and from Friday 4PM until Sunday afternoon. Throughout testing, which was performed 7 days a week, rats were given free access to water from 4-5PM each day. The rats were maintained so that their body weight dipped no lower than 85% of their free-drinking weight. The "Handbook of Laboratory Animal Management and Welfare" (Wolfensohn and Lloyd 1998) was followed and all procedures conformed to the United Kingdom Animals (Scientific Procedures) Act (1986).

### APPARATUS

Rats were trained in 60cm x 74cm x 55cm sound-attenuating chambers (Med Associates Inc., St Albans, VT) fitted with ventilation fans, opaque viewing windows and video cameras. A Perspex testing cage (34mm x 29mm x 25mm) with metal bar floors and slots for modular testing equipment was located inside the sound-attenuating shell. A retractable lever and liquid reward spigot were located on the left wall of each chamber. The reward spigot was recessed in a custom-built reward magazine, the faceplate of which had a narrow opening designed to allow for accurate measurements of licking behaviour via an electronic contact lickometer (Weijnen 1989). A piezoelectric sounder (2900Hz, 85dB) and a white LED (approximately 2072 mcd luminosity) were located in the interior of this reward magazine.

Liquid rewards were delivered at a rate of 0.05ml/sec by each of two computer controlled syringe pumps (model PHM - 100, Med Associates Inc., St Albans, VT) that dispensed liquid from 50 ml glass syringes (Rocket, London) with stainless steel plungers. One of these syringes dispensed distilled water while the other dispensed 0.5% w/v sodium saccharine solution (when activated simultaneously, the solution from the two pumps mixed at the reward spigot resulting in a 0.25% w/v saccharin solution). The syringes were connected via an 18-gauge needle to a reward spigot by Teflon tubing. The stiffness of the glass syringes, the stainless steel plungers and the tubing prevented the pressure waves produced by the pumps from being attenuated and produced precisely timed rewards with reliable flow rates. The tubing from the pumps was connected to a tube-within-a tube arrangement with saccharin flowing through the inner tube and tap water flowing through a gap between the inner and outer tubes. This provided mixing of the water and the saccharine solution. The cross-sectional area of the inner tube and the annular gap between the inner and outer tubes were equivalent to allow for equal flow rates of the two liquids.

Computerized behavioural testing was conducted by the MED-PC™ data experimental control system (Med Associates Inc., St Albans, VT). Summary measures of the rats' performances were displayed on-line and could be viewed in conjunction with the video signals taken from each box. The temporal resolution of the system was 2msec. All behavioural events were timestamped with this resolution and then these data were analyzed in the programming language AWK (Thompson Toolkit, Thompson Automation) to reconstruct final summary data for each trial. The trial data were incorporated into a database that was subsequently analyzed by Microsoft Excel 2000 ™ and SPSS 10.0 for Windows™.

PROCEDURES

Rats were advanced through the following stages as a group when they were judged, by visual inspection, to be responding at asymptotic levels of performance.

*STAGE 1: REWARD MAGAZINE TRAINING*

The rats were trained in three 45-minute sessions using the following procedure: the session was started when the outer doors of the sound-attenuating chamber were closed and was ended when they were opened. When the animal first licked the reward spigot following trial onset (unsignaled to the rat) there was a variable delay of 0.1, 0.2, 0.4, or 0.8s (pseudorandomly chosen at the start of each trial) followed by a fixed delay of 0.5s and subsequent presentation of a discriminative stimulus (the primary reinforcer signal) indicating to the rats that they could lick for the primary reinforcer (this stimulus was functionally equivalent to animals seeing or hearing the delivery of food pellets within second-order schedules of food reinforcement carried out previously, e.g. Katz (1979)). The rats were divided into two groups of different primary reinforcer signal modality. The primary reinforcer signal for one group was the presentation of a brief tone using the piezoelectric sounder located inside the reward magazine, for the other group the presentation of a brief light using a white LED also located inside the reward magazine. If a lick was made between 0.5s and 2s of the presentation of this primary reinforcer signal the animal received 0.4ml saccharin solution lasting four seconds (the primary reinforcer signal remained on during this time). When the lick bout following primary reinforcement ended (defined as an inter-lick interval greater than 300ms) the trial ended (unsignaled to the rat) and the next trial began immediately. If no lick was made in the presence of the primary reinforcer signal then the signal was turned off, an error was recorded, and there was timeout period of 5 seconds (unsignalled to the rat) before the trial ended.

*STAGE 2: MODIFIED FR1 TRAINING*

All rats were then trained to press a lever for the primary reinforcer on a modified FR1 schedule for four daily sessions each lasting 45 minutes. One bar press led

to the onset of the second-order stimulus (depending on the primary reinforcer signal modality group this stimulus was either the light or the tone but was a different modality from the primary reinforcer signal) lasting 0.5s followed by the simultaneous presentation of both the primary reinforcer signal and second-order stimulus. If a lick was then made in the following five seconds 0.4ml of saccharin solution was presented. Following primary reinforcement both stimuli were then switched off, and the next trial started immediately. If no lick was made within 5 seconds of the primary reinforcer signal onset, an error was recorded, the primary reinforcer signal and second-order stimulus were switched off and the bar was retracted for a 2 second timeout.

*STAGE 3: SECOND-ORDER SCHEDULE TRAINING*

The first two stages of second-order schedule training each took four daily 1-hour sessions. In the first stage the number of bar presses required to earn primary reinforcement was increased to 5. After each bar press the second-order stimulus was presented for 0.5 seconds. This second-order schedule can be written FR5 (FR1: S) to indicate that 1 bar press resulted in the second-order stimulus (FR1: S) and that 5 second-order stimuli were required to earn primary reinforcement (FR5). The procedures for the presentation of second-order stimuli and the delivery of the primary reinforcer were the same as those used during modified FR1 training. However, when an error was made the schedule was not restarted. Instead, one more bar press was required to repeat the presentation of the second-order stimulus and primary reinforcer signal, allowing the animal to lick for primary reinforcement. During the second stage of second-order schedule training the number of bar presses required to present the second-order stimulus was increased to 5, namely FR5 (FR5: S). In the final stage of training the FR5 (FR5: S) was modified to a FI 4min (FR10: S) second-order schedule. This meant that 10 bar presses brought the second-order stimulus and that the first FR10 schedule initiated and completed after a fixed interval of 4 minutes (FI 4min) resulted in delivery of primary reinforcement (see Figure 2.1). Bar presses made during the presentation of the second-order stimulus were not counted in the

second-order schedule. In this final phase the session length was increased to 90 minutes. This regime is similar to that used in most rat second-order schedule studies (see Everitt and Robbins (2000) for a review).

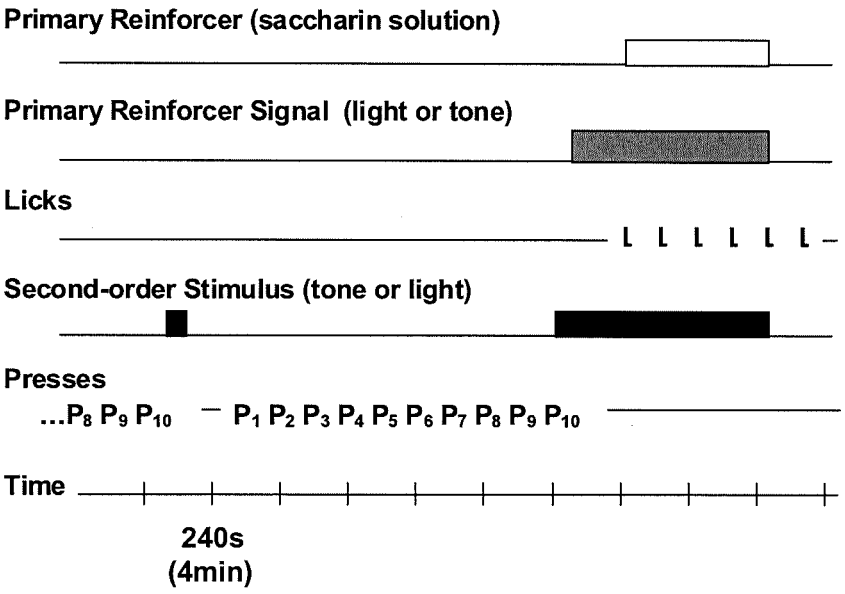



*Figure 2.1* Schematic example of the FI 4min (FR10: S) second-order schedule. From bottom: Time line where tick mark represents every two seconds towards the end of the FI 4min component of the second-order schedule of reinforcement. Presses line where $P_x$ represents a press with 'x' the number of presses in the sequence. After 10 presses are made ($P_{10}$) before 4 minutes has elapsed the second-order stimulus (black block) is presented for 0.5s. 10 presses made after the FI4minutes results in the presentation of the second-order stimulus for 0.5s followed by the simultaneous presentation of the second-order stimulus and the primary reinforcer signal (black and grey blocks). Once a lick is made primary reinforcement is delivered for 4 seconds (white block). All stimuli are then switched off.

*STAGE 4: OMMISSION TEST*

Following nine days of baseline training the second-order stimuli were omitted. However, this data has not been shown since there were no significant effects of omitting the second-order stimulus. Following another five training days until rats reached baseline responding levels, as defined by visual inspection, the animals were then tested again during second-order stimulus omission (FI 4min (FR10)). This second-order schedule was identical to before with the exception that the

second-order stimuli that were originally presented following the FR10 schedule prior to completion of the FI 4min schedule were omitted. Bar presses made during the time when the second-order stimulus would have occurred were not counted in the second-order schedule. On testing day 1 the second-order stimulus was presented, on days 2,3 and 4, it was omitted, and on days 5 and 6, it was reinstated.

DATA ANALYSIS

Repeated-measures polynomial contrasts were performed to determine if patterns of responding during the second-order stimulus omission test best matched a linear, quadratic or cubic function with repeated measures factor *Day* (reflecting days 5-9 of the second-order stimulus omission test) and between-group factor *Primary Reinforcer Signal Modality*. We initially hypothesized a U-shaped pattern with low responding during second-order stimulus omission.

Repeated-measures ANOVA's were individually performed on both the average bar presses made per fixed interval and the percentage of second-order stimuli followed by a lick at the reward spigot within 2 seconds per fixed interval. Results from days in which the second-order stimulus was present (second-order stimulus (+)) were compared to when the second-order stimulus was omitted (during second-order stimulus omission the second-order stimulus was defined as the time in the schedule where the second-order stimulus would have occurred had it been presented (second-order stimulus (-))).

The effects of checking and licking the reward spigot on bar-pressing were independently tested using ANCOVA (the most appropriate test as outlined by (Bland and Altman 1995)) where the dependent variable was the average number of presses prior to the fixed interval per rat per day, the random factor was *Subject*, and the covariate was either the average percentage of second-order stimuli followed by a lick per fixed interval per rat per day (*Checking*) or the

average number of licks prior to the fixed interval per rat per day (*Licking*), respectively. The correlation coefficient "B" provided an estimate of the change in the bar-pressing that could be attributed to a change of one unit of the potentially competing response of checking for primary reinforcement. All analyses were performed using Microsoft Excel 2000™ and SPSS 10.0 for Windows™.

**RESULTS**

Contrary to our expectations, the average rate of bar-pressing within each interval was *increased* during omission of the second-order stimulus (41.06 presses/fixed interval (± 6.16 95% C.I.), days 6-8) and subsequently *decreased* when second-order stimuli were reinstated on days 9 and 10 (32.97 presses/fixed interval (± 9.03 95% C.I.). This was confirmed statistically by a polynomial contrast (see Figure 2.2). ANOVA revealed a significant 24% increase in average bar-pressing rates between the testing days in the absence of the second-order stimulus (days 6,7,8) versus those during which the second-order stimulus was presented (days 5,9,10) ($F_{(1,7)}$=43.328, p<0.001).

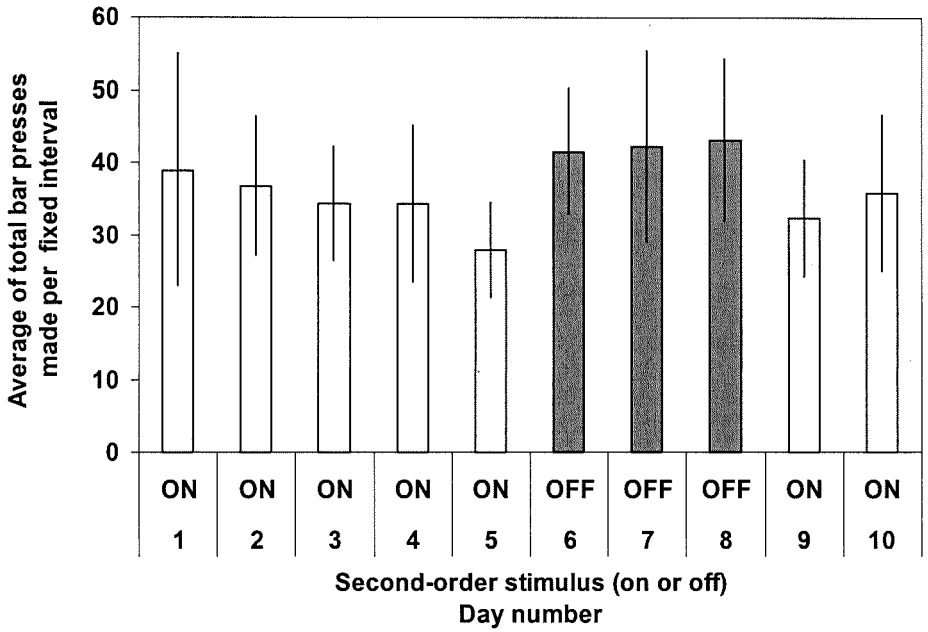



*Figure 2.2* Average bar presses per fixed interval during baseline training (days 1-4) and testing (days 5-10) (Error bars ± 95% confidence intervals). A repeated measures polynomial contrast performed on days 5-9 revealed the pattern of results was best described by a quadratic function with an inverted U-shape ($F_{(1,6)}$=23.991, p=0.003) with responding lowest during baseline and second-order stimulus reinstatement and highest during second-order stimulus omission. No significant *Primary Reinforcer Signal Modality* main effect or interaction was found. N=8 rats.

We considered the possibility that the second-order stimulus was not associated with the primary reinforcer. We rejected this possibility when we found that rats were more likely to lick within two seconds of presentation of the second-order stimulus when the second-order stimulus was presented (second-order stimulus (+)) than when it was omitted (second-order stimulus (-); licks were measured relative to when the second-order stimulus would have occurred had it not been omitted), as confirmed by a polynomial contrast (see Figure 2.3). ANOVA revealed a significant 93% decrease in the average percentage of second-order stimuli followed by a lick (second-order stimuli immediately followed by primary reinforcement were excluded) between the testing days in the absence of the second-order stimulus (days 6,7, 8) versus those during which the second-order stimulus was presented (days 5,9,10) ($F_{(1,23)}$=64.529, p=0.001).

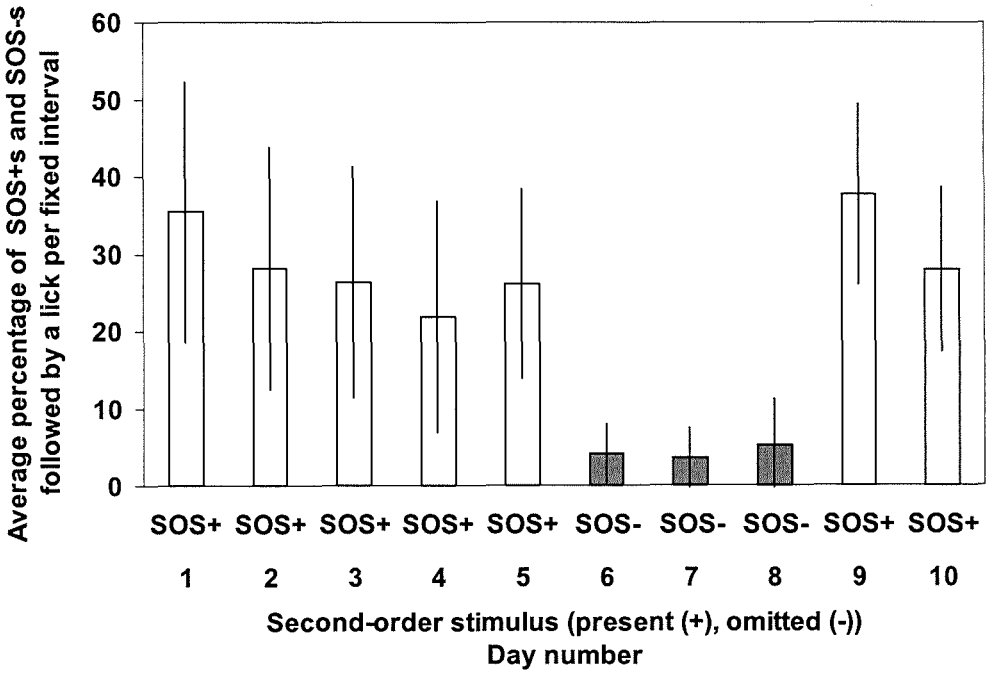



*Figure 2.3* Average percentage of second-order stimuli (+) (SOS+) and second-order stimuli (-) (SOS-) followed by a lick per fixed interval during baseline training (days 1-4) and second-order stimulus omission test (days 5-10). Second-order stimuli immediately followed by primary reinforcement were excluded (Error bars ± 95% confidence intervals). A repeated measures polynomial contrast performed on days 5-9 revealed the pattern of results was best described by a U-shaped quadratic function ($F_{(1,6)}$=29.437, p=0.002). No significant *Primary Reinforcer Signal Modality* main effect or interaction was found. N=8 rats.

We tested the hypothesis that licks in response to the second-order stimulus competed against bar-pressing, thereby having a paradoxical effect of supressing overall bar-pressing rates when the second-order stimulus was present. It was found that the percentage of second-order stimuli followed by a lick was not related to the rate of bar-pressing (see Figure 2.4). Indeed, there was a positive relationship between the average rates of pressing and licking during the second-order schedule (see Figure 2.5).

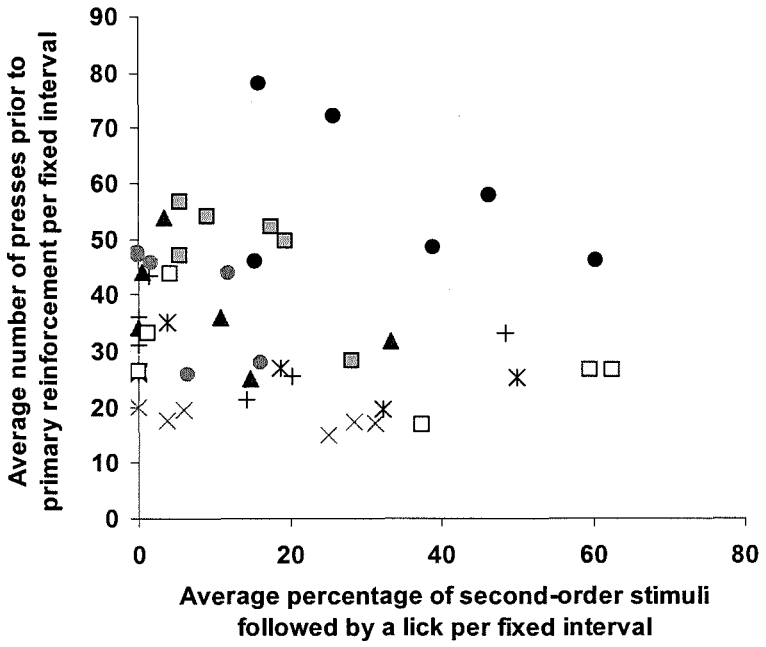



*Figure 2.4* Scatterplot of average number of presses prior to primary reinforcement per fixed interval versus average percentage of second-order stimuli followed by a lick per fixed interval. Responses to second-order stimuli that were immediately followed by the primary reinforcer were excluded. Each point corresponds to the value for a given test day (days 5-10) for a given subject, with unique marker points for each subject. ANCOVA revealed a significant effect of *Subject* on bar-pressing ($F_{(5,41)}$=3.798, p=0.001) but no overall effect of *Checking* on bar pressing (correlation co-efficient "B"=0.028, $F_{(1,41)}$=0.062, p=0.805).

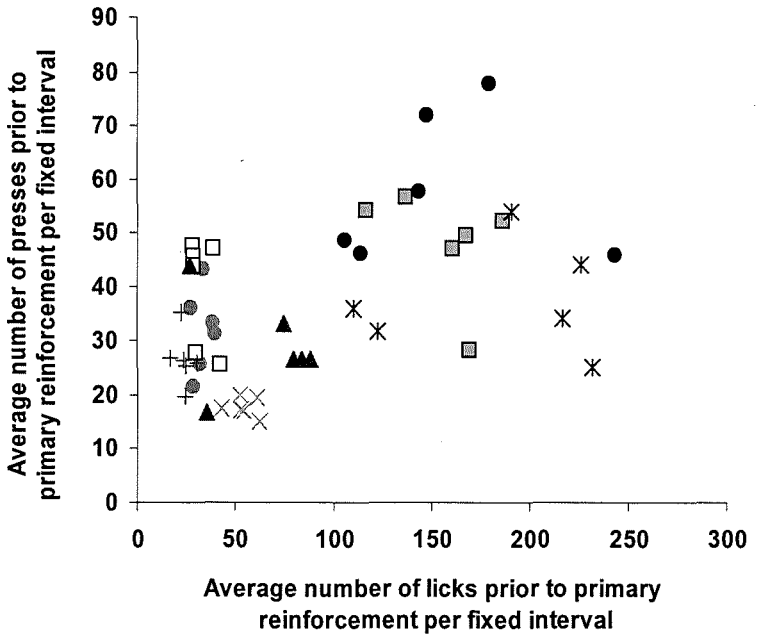



*Figure 2.5* Scatterplot of average number of presses prior to primary reinforcement per fixed interval versus average number of licks prior to primary reinforcement per fixed interval. Each point corresponds to the value for a given test day (days 5-10) for a given subject, with unique marker points with subject. ANCOVA revealed a significant effect of *Subject* on bar-pressing ($F_{(5,41)}$=4.768, p=0.002) and a significant positive relationship of *Licking* on bar pressing (correlation co-efficient "B"=0.107, $F_{(1,41)}$=16.385, p<0.001).

We then sought to determine how the second-order stimuli affected the temporal pattern of responding within the second-order schedule. As shown in Figure 2.6, bar-pressing was uniformly high when the second-order stimulus was omitted. In contrast, when the second-order stimulus was present, the rats' pattern of bar-pressing increased prior to the presentation of the second-order stimulus and decreased in the following 3 sec thereafter. Thus, although second-order stimuli influenced the temporal pattern of responding in this experiment, they did not *enhance overall* response rates.

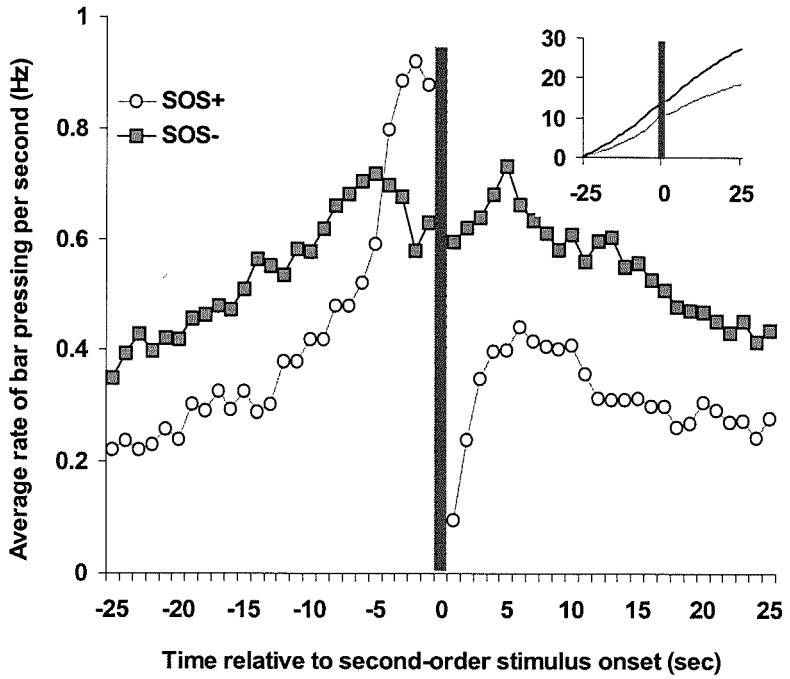



*Figure 2.6* Average bar-pressing rate (Hz) per second-order stimulus made relative to second-order stimulus onset during normal testing (open circles; SOS+) and during second-order stimulus omission (grey blocks; SOS-). Responses to the second-order stimuli that were immediately followed by the primary reinforcer were excluded. Bin size= 1 second. N=8 rats. *N.B. Data at 0 seconds were excluded (indicated by grey bar), since every second-order stimulus was immediately preceded by at least one bar press.*

*Inset (top right):* Representation of same data as histogram except average *cumulative* rates of bar-pressing (Hz). Second-order stimuli present (grey line), second-order stimuli absent (black line). X-axis: Time relative to second-order stimulus onset; y-axis: Average cumulative rate of bar-pressing per second (Hz). *N.B. Data at 0 seconds were excluded (indicated by grey bar), since every second-order stimulus was immediately preceded by at least one bar press.*

## DISCUSSION

In this study response rates in a second-order schedule for sweetened water reinforcement were significantly increased during omission of the second-order stimulus, in contrast to most previous reports (Arroyo et al. 1998; Di Ciano and Everitt 2003a; Goldberg et al. 1979; Goldberg and Tang 1977; Katz 1979; Kelleher 1966). It was confirmed that this effect occurred even though second-order stimuli were associated with the primary reinforcer (the second-order stimuli triggered the rats to check for the primary reinforcer) and even though these responses did not compete with bar-pressing. Importantly, response rates increased prior to the second-order stimulus and decreased for several seconds thereafter. This temporal pattern of responding was not found during second-order stimulus omission in which response rates were consistent throughout the schedule.

The design of this second-order schedule differed from many studies (see Everitt and Robbins 2000). As the saccharin solution could not be stored for collection by the rat and was not easily visible, the rat was required to make a response at the reward spigot within a certain time of the second-order stimulus to receive the primary reinforcer. It was anticipated that checking at the reward spigot might have confounded the hypothesized enhancement in bar-pressing. In an attempt to minimize checking, we used an explicit primary reinforcer signal (a discriminative stimulus for licking indicating when primary reinforcement was available (see Methods)). However, the addition of this primary reinforcer signal does not explain the unexpected increase in responding during second-order stimulus omission for the following reasons. Firstly, the second-order stimulus was still associated with the primary reinforcer, as shown by licks in response to the second-order stimulus, and these second-order stimuli also had an effect on the temporal pattern of bar-pressing. Additionally, previous second-order schedules using food or sex for reinforcement also implicitly contained a primary

reinforcer signal, as the subjects almost certainly heard or saw the primary reinforcer arrive.

The temporal pattern of responding that we found around the second-order stimulus is indicative of response patterns found within fixed-interval schedules of primary reinforcement. This is characterized by low response rates in the early stages of the fixed-interval, followed by positively accelerated responding prior to primary reinforcement. Response rates are inhibited immediately after the primary reinforcer since the delivery of primary reinforcement signals a subsequent period of non-reinforcement (Ferster and Skinner 1957). Second-order stimuli maintaining this pattern of responding could have resulted in the decrease in overall response rates seen here, since it has previously been demonstrated that wheel running rates of rats can be lower over a fixed-interval schedule of primary reinforcement compared to during an equivalent period of unreinforced running (Skinner and Morse 1958). This supports previous conclusions that measures of temporal patterns of responding are vital in providing an accurate measure of the effects of second-order stimuli on operant responding (Gollub 1977; Kelleher 1966; Stubbs 1971).

Most second-order schedule studies report similar temporal patterns of responding as shown here but find a decrease in overall response rates during second-order stimulus omission (Everitt and Robbins 2000; Schindler et al. 2002). However, both Byrd and Marr (1969) and Stubbs (1971) using pigeons found positively accelerated responding to second-order stimulus presentation followed by pausing *and* increased overall response rates during second-order stimulus omission. In fact the pattern of results reported here might be more common than the literature indicates, as the second-order stimulus omission test has only been performed in six out of the thirty-four rat second-order schedule studies (Alderson et al. 2000b; Arroyo et al. 1998; Di Ciano and Everitt 2003a; Everitt et al. 1989; Everitt et al. 1987; Parkinson et al. 2001; Weissenborn et al. 1997). Furthermore, from these, Weissenborn et al. failed to find significant

differences in overall rates of responding during second-order stimulus omission within a second-order schedule of cocaine reinforcement, and Alderson et al. reported significant decreases in bar-pressing only during the first interval of second-order stimulus omission within a second-order schedule of heroin reinforcement. In this regard, we found no significant differences from ANOVA between average bar-pressing in the first interval versus the remaining intervals of the second-order schedule.

However, there is still no way to predict which schedule parameters produce more or less rate-enhancing effects. For instance, many similar schedules to the one employed here, such as the FI 15min (FR10: S) used often with rats (Everitt and Robbins 2000); a FI 5min (FR10: S) for cocaine used with squirrel monkeys (Goldberg et al. 1981); and a FR15 (FI 4min: S) for food used with pigeons (Kelleher 1966) have shown a reduction in operant responding during second-order stimulus omission. There is a possibility that even though saccharin reinforced operant responding and produced conditioning effects, it may not have been powerful enough to impart rate-enhancing properties to the second-order stimulus. This may also provide an explanation for omission effects varying with reinforcer type with stronger effects reported for cocaine compared to food, (Goldberg et al. 1981; Spear and Katz 1991) and relatively weak effects reported for heroin (Alderson et al. 2000b). Again, however, this explanation is not consistent with studies showing reductions in overall operant responding during second-order stimulus omission using other natural rewards (Everitt et al. 1987; Katz 1979). Therefore, there is no clear explanation for our anomalous findings or those of Stubbs (1971) and Byrd and Marr (1969).

Consequently, it is becoming increasingly less certain that reductions in overall responding within second-order schedules reflect a reduction in the control second-order stimuli have on maintaining reward/drug-seeking behaviour (e.g., (Backstrom and Hyytia 2003; Evans et al. 2003; Lee et al. 2003; Negus and Mello 2003; Platt et al. 2003; Semenova and Markou 2003). Indeed, our results

suggest potential reductions in overall second-order schedule responding resulting from experimental manipulations, such as administration of anti-addiction vaccines, could be indicative of the second-order stimuli imposing stronger effects on the psychological processes underlying responding. It would be beneficial, therefore, for future studies considering the brain processes underlying reward/drug-seeking to measure overall response rates and the pattern of responding in the presence versus absence of the second-order stimulus and compare these effects to the effects of a biological manipulation.

## CHAPTER 3

# NUCLEUS ACCUMBENS NEURONS IN THE RAT EXHIBIT DIFFERENTIAL ACTIVITY TO CONDITIONED REINFORCERS AND PRIMARY REINFORCERS WITHIN A SECOND-ORDER SCHEDULE OF SACCHARIN REINFORCEMENT

The work presented in this chapter has been published previously
(Wilson and Bowman, 2004, European Journal of Neuroscience 20: 2777-2788).

**ABSTRACT**

The nucleus accumbens has been associated with processing information related to primary reinforcement and reward. Most neurophysiological studies report that nucleus accumbens neurons are phasically excited to the onsets of salient events during the seeking of reinforcement and to the delivery of primary reinforcers. However, a minority of studies reports inhibition during primary reinforcement. We recorded from sixty-five neurons in the nucleus accumbens whilst thirsty rats performed under a second-order schedule of saccharin reinforcement. This allowed us to analyse neural activity and behaviour during reinforcer-seeking in the presence of conditioned reinforcers (second-order stimuli, also called *conditioned stimuli*), and during primary reinforcer consumption. Specifically, we sought to examine the valence of potential neural responses to primary reinforcement, to compare these responses to second-order stimulus evoked responses, and to determine if responses were differential to second-order stimuli presented at different time points within the schedule. Fifty out of sixty-five neurons we sampled responded to the second-order stimulus and/or consumption of the primary reinforcer. Most neurons in our sample exhibited excitation following the second-order stimulus and inhibition to the primary reinforcer, a pattern also present over the average response of the neural population. However, there was no systematic variation on neural responses evoked by second-order stimuli presented at different temporal proximities to primary reinforcement. Our results provide evidence that partially overlapping mechanisms within the nucleus accumbens differentially process conditioned reinforcers and primary reinforcers.

## INTRODUCTION

The nucleus accumbens, a brain region comprising most of the ventral striatum, has been viewed classically as an interface between motivation and action (Mogenson et al. 1980). It forms an integral part of the mesolimbic dopamine system that has been extensively implicated in reward and reinforcement (Kelley and Berridge 2002; Robbins and Everitt 1996; Schultz 1998; Wise 1982). Current theories propose that dopamine neurotransmission in the nucleus accumbens is involved in incentive motivation, or "wanting" of primary reinforcement (Berridge and Robinson 1998; de Borchgrave et al. 2002; Salamone and Correa 2002; Wyvell and Berridge 2001). Recent work has extended these theories by demonstrating that the nucleus accumbens contributes to flexible approach responses to rewarding stimuli or away from aversive stimuli (Horvitz 2000; Ikemoto and Panksepp 1999; Setlow et al. 2003). In contrast, opioid receptor activation within the nucleus accumbens has been implicated in mediating "liking" but not "wanting" of primary reinforcement (Kelley et al. 2002; Zhang et al. 2003). Thus, within the nucleus accumbens two psychologically distinct processes are probably represented in the activity of its neurons.

Most neurophysiology studies have found that single neurons in the nucleus accumbens are phasically excited to the delivery of a primary reinforcer and to salient events occurring during seeking of reinforcement (Bowman et al. 1996; Carelli et al. 2000; Cromwell and Schultz 2003; Hassani et al. 2001; Schultz et al. 1992; Shidara et al. 1998; Tremblay et al. 1998). However, some authors report that more neurons are inhibited than excited to reinforcement delivery (Chang et al. 1998; Nicola et al. 2004c; Peoples and West 1996). We recorded from single nucleus accumbens neurons whilst rats performed on a second-order schedule of saccharin reinforcement. This behavioural task allows neural activity during reinforcer-seeking and reinforcer-taking to be assessed (Everitt and Robbins 2000), since the operant response is maintained by both the primary reinforcer and intermittent presentations of conditioned reinforcers (second-order stimuli,

also can be called *conditioned stimuli*) (Alderson et al. 2000b; Arroyo et al. 1998; Byrd and Marr 1969; Goldberg et al. 1979; Goldberg and Tang 1977; Katz 1979; Kelleher 1966; Parkinson et al. 2001; Pears et al. 2003; Schindler et al. 2002; Stubbs 1971). We targeted the electrode at the nucleus accumbens core since lesions of the core, but not shell, impaired rats' ability to learn a second-order schedule of heroin reinforcement (Hutcheson et al. 2001).

Using this behavioural paradigm, we wanted to answer the following questions: (1) Are nucleus accumbens neurons excited or inhibited by primary reinforcement given the equivocal findings in previous work? (2) Since second-order stimuli are considered to be reinforcers of behaviour (Everitt and Robbins 2000; Kelleher 1966), do nucleus accumbens neurons fire similarly to second-order stimuli and to primary reinforcement? (3) As it has been demonstrated that activity in the nucleus accumbens neurons varies systematically with the presentation of discriminative stimuli that signal different levels of work required to earn primary reinforcement (Bowman et al. 1996; Shidara et al. 1998), will neurons fire differentially to early second-order stimuli, which are unlikely to be followed by primary reinforcement, *versus* late second-order stimuli, which are more likely to be followed by primary reinforcement?

## METHODS

### SUBJECTS

Eight adult male Lister Hooded rats (Harlan UK) that were used in a previous study (Wilson and Bowman 2004b) were housed on a light 12h: dark 12h light cycle in quadruplets during behavioural training and in isolation during neurophysiological testing. During behavioural training and neurophysiological testing rats were provided with access to water from 4-5PM on each weekday and from Friday 4PM until Sunday afternoon. During the second-order stimulus omission test described below, rats were given access to water from 4-5PM every day. Rats weighed 378-487g at the start of training and were maintained so their body weight dipped no lower than 85% of this free-drinking weight. This water restriction regime allowed the animals to continue to grow throughout the testing period. The "Handbook of Laboratory Animal Management and Welfare" (Wolfensohn and Lloyd 1998) was followed and all procedures conformed to the United Kingdom 1986 Animals (Scientific Procedures) Act.

### APPARATUS

#### *BEHAVIOUR*

Rats were trained in Perspex testing cages (34cmx29cmx25cm) situated inside sound-attenuating chambers (60cmx74cmx55cm) (Med Associates Inc., St Albans, VT). A retractable lever and reward magazine were located on the left wall of each chamber. A piezoelectric sounder (2900Hz, 85dB), a white LED (approximately 2072 mcd luminosity) and a liquid reward spigot that was connected to an electronic contact lickometer were all located in the interior of the reward magazine. Sodium saccharin solution (0.25% w/v) was delivered through the reward spigot by two computer controlled syringe pumps (model PHM - 100, Med Associates Inc., St Albans, VT).

#### *NEUROPHYSIOLOGY*

Custom-built electrode arrays each contained a movable bundle of four 50μm stainless steel microwires coated in Teflon (tip impedance 0.5-1.5MΩ) that were of a similar length. The bundle of 4 wires could be advanced, but not retracted, by turning an 80-thread per inch set screw (Small Parts Inc., Miami Lakes, Florida), within which one turn advanced the electrode approximately 317.5 μm. The array measured approximately 11mm along the anteroposterior and 6mm mediolateral axis, and each array weighed approximately 1.3g. During post-surgery testing, the subject was attached to a preamplifier headstage that used field effect transistors (FETs, input impedance 100MΩ). This headstage was located within one of the behavioural testing chambers and was attached via a flexible cable to an electrical commuter (Stoelting Co., Wood Dale, Illinois). Neural activity was recorded differentially from each of 2 pairs of wires to reduce noise, movement, and lickometer artefacts (Sasaki et al. 1983). Lickometer artefacts were also minimised by the use of a custom-built lickometer (Malcolm McCandless, University of St Andrews). This lickometer produced alternating current, which meant artefacts were of a high enough frequency (>5000Hz) to be filtered out.

Using a Neurolog System™ (Digitimer Research Instrumentation, Hertfordshire, UK) the differential voltage signal from each of the two pairs of wires was amplified by 100,000x (AC coupling) and passed through a series of filters to attenuate frequencies <1000Hz and >5000Hz. Fifty Hz noise was eliminated using two Quest Scientific™ 'Hum Bug' digital filters (Digitimer Research Instrumentation, Hertfordshire, UK). The activity was then processed by a CED 1401™ data acquisition system (Cambridge Electronic Design, Cambridge, UK). Behavioural events were timestamped in parallel with the neural activity by the CED system through digital input pulses generated by the MED-PC™ (Med Associates Inc., St Albans, VT). Thus, although the rate at which data were sampled on the CED™ system was 20kHz, the resolution for timestamping behavioural events was limited to that of the MED-PC™ system (2ms). Spikes were detected and anlaysed on-line using a Hameg Manual oscilloscope (model

HM203-7, Hameg Ltd., Luton, UK) and a template sorting algorithm of the commercial software package Spike 2™ (version 5.01; CED, Cambridge, UK).

## PROCEDURES

### *BEHAVIOURAL TRAINING*

The training of the rats has been described in detail previously (Wilson and Bowman 2004b). Briefly, rats progressed through the following stages of training when it was decided by visual inspection that the group was responding at an asymptotic level of performance.

*Stage 1: Reward magazine training.* Rats were trained in three 45-minute sessions to lick the reward spigot to obtain primary reinforcement. When the rat made a lick there was a variable delay of 0.6, 0.7, 0.9, 1.3s (pseudorandomnly chosen on each trial) followed by the presentation of a discriminative stimulus, the "reinforcement signal". Rats were divided into two groups where the reinforcement signal for one group was a tone using a piezoelectric sounder, and for the other was a light from a white LED. If a lick was made 0.5s-2s after the presentation of this signal, 0.4ml (0.1ml/s) sodium saccharin solution (0.25% w/v) was delivered paired with the continued presentation of the reinforcement signal. Since there was no bowl for collecting the saccharin solution, rats consumed the primary reinforcer directly at the spigot during its delivery. If no lick was made within 2s of the reinforcement signal then an error was recorded, and the trial restarted. A 'correct' trial was ended when the lick bout following primary reinforcement ended (defined as an inter-lick interval greater than 300ms).

*Stage 2: Modified FR1 training.* Rats were then trained to press a lever for primary reinforcement on a modified FR1 schedule for four 45-minute daily sessions. After one bar press the second-order stimulus was presented for 0.5s, followed by simultaneous presentation of the second-order stimulus and the reinforcement signal (the second-order stimulus was a tone or a light but was not

the same as the reinforcement signal). Saccharin solution was then delivered as in stage 1 with the exception that a lick between 0.5s and 5s following presentation of the reinforcement signal led to a 'correct' trial and error trials were followed by a timeout period during which the bar was retracted for 2s.

*Stage 3: Second-order schedule training.* Rats were then trained for four 1-hour daily sessions to press five times to complete the schedule with each press followed by a 0.5s presentation of the second-order stimulus, which can be written *FR5 (FR1: S)*. Subsequent stimuli presentation and reinforcement delivery was the same as in stage 2, except that when an error was made no timeout period or bar retraction followed, with the next bar press earning reinforcement. For the next four 1-hour daily sessions the number of bar presses required to present the second-order stimulus was increased to 5, i.e. FR5 (FR5: S). The final stage of training was nine daily 90-minute sessions on a FI 4min (FR10: S) second-order schedule. This meant that for every 10 bar presses made prior to the fixed interval of 4 minutes (FI 4min) a second-order stimulus was presented, and the first FR10 schedule initiated and completed after the FI 4min brought the *terminal second-order stimulus*, following which the schedule was completed and reinforcement delivered (see Figure 3.1). Bar presses made during the second-order stimulus presentation did not count towards second-order schedule responding. These training procedures are comparable to those in previous rat second-order schedule studies (see Everitt & Robbins, 2000, for a review).

*Stage 4: Omission test.* Once rats reached baseline responding levels as defined by visual inspection, the animals were then tested during second-order stimulus omission (FI 4min (FR10)). This second-order schedule was the same as the FI 4min (FR10: S) used in training except all second-order stimuli that did not terminate the schedule were omitted. Bar presses within the time window when the second-order stimulus would have occurred did not count towards second-order schedule responding. Since there was no effect of omission (data not shown) the rats were trained to baseline levels again and re-tested during

second-order stimulus omission. On testing day 1 the second-order stimulus was presented, on days 2,3 and 4, it was omitted, and on days 5 and 6, it was reinstated.

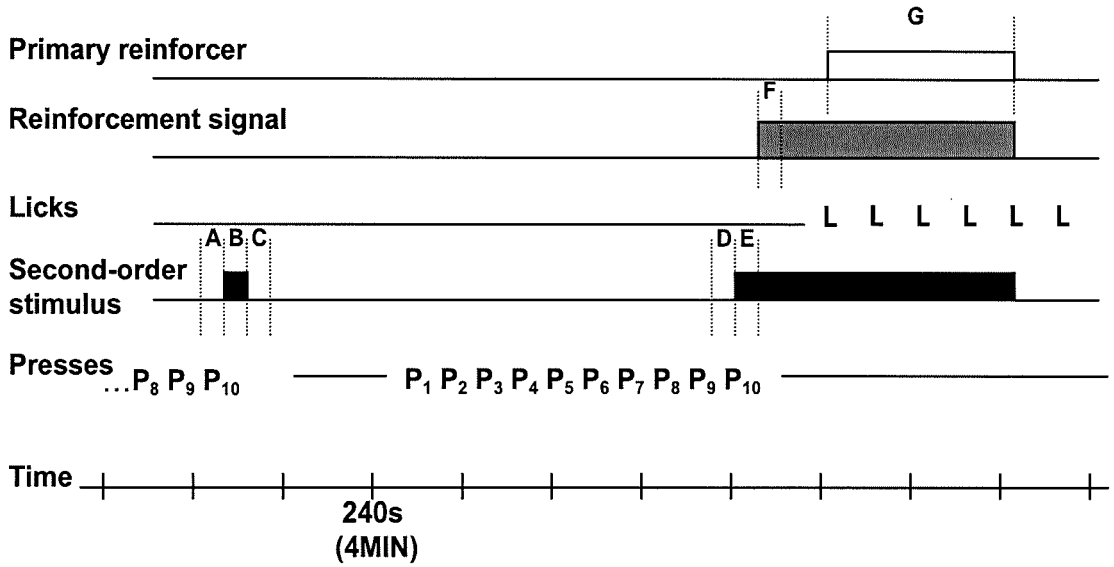



*Figure 3.1* Schematic representation of FI 4min (FR10: S) second-order schedule with time windows for spike counts. 10 presses (P) made prior to 4-minute interval resulted in second-order stimulus presentation that was not followed by the reinforcement signal (small black box). 10 presses after FI 4min produced the terminal second-order stimulus (large black box) that was subsequently paired with the reinforcement signal 0.5s later (grey box). A subsequent lick (L) 0.5-5s after this compound stimulus resulted in primary reinforcement (white box). All stimuli were then switched off. The next trial was started when the lick bout following primary reinforcement ended (when the inter-lick interval was greater than 300ms). A,D: **Baseline** time window –0.5-0s before second-order stimulus onset; B,E: **Second-order stimulus onset** time window 0-0.5s after second-order stimuli onset; C,F: **Second-order stimulus offset** time window 0-0.5s after second-order stimulus offset; G: **Primary reinforcement** time window 0-4s after primary reinforcer onset.

*SURGERY*

Following behavioural training and testing, rats underwent surgery whereby an electrode array was implanted onto the skull. Initially, animals were anaesthetized with a mixture of halothane (5% for induction, 1.5% for

maintenance) and oxygen (1.0-1.2 l/min). A pre-surgical nonsteroidal, nonopiate analgesic Rimadyl™ (0.1ml/kg; 5% w/v carpofen; Pfizer Ltd., Kent, UK) was injected subcutaneously. The top of the animal's head was shaved and a midline incision was made on the scalp. Connective tissue on the dorsal surface of the skull was then retracted by blunt dissection. The hole drilled for the electrodes was situated stereotaxically in the nucleus accumbens core area (anteroposterior, +1.7mm; mediolateral, +1.5mm lateral; dorsoventral, -6.0mm (approximate length of guide tube relative to skull surface), relative to bregma). Holes positioned just outside the perimeter of the electrode array were drilled and tapped for 4-6 retaining screws (0-80 hex head set screws, 1/4 inch; Small Parts, Miami Lakes, Florida). The electrode array was lowered in place using the stereotaxic arm and dental acrylic was used to attach the array and the skull screws to the cranium. Vicryl™ absorbable sutures were used to close the incision.

*NEUROPHYSIOLOGICAL RECORDING*

We recorded successfully from four out of the eight rats reported in Wilson & Bowman, 2004. Each rat was given 5-7 days to recover from surgery. Neural recording lasted approximately three to four weeks during which neural activity was recorded whilst animals performed on the second-order schedule of reinforcement FI 4min (FR10: S). Three of these rats received presentations of the tone for the second-order stimulus, whilst the other received presentations of the light.

*HISTOLOGY*

Following neurophysiological recording rats were killed by overdose with 0.7ml Dolethal™ (200 mg/l pentobarbitone sodium BP; Univet Ltd., Oxford, UK) and perfused intracardially with 0.1% phosphate buffer saline followed by a fixative (4% paraformaldehyde in 0.1M phosphate buffer). Following perfusion, the brains were left in the cranium to steep in the fixative for approximately 30 minutes, removed, and then placed in sucrose solution (20% sucrose in 0.1M phosphate

buffer) overnight. 50-µm thick sections were cut on a freezing microtome collected in 0.1M phosphate buffer and 1:8 sections stained for Nissl bodies. Stained sections were then analysed under a light microscope and damage from electrode tracts were mapped onto standardised sections of the brain (Paxinos and Watson 1997).

## DATA ANALYSIS

### *BEHAVIOUR*

A histogram of the average bar-pressing rate (Hz) relative to the onset of second-order stimuli that did not terminate the schedule during the omission test (Wilson and Bowman 2004b) and neurophysiological recording was created (during second-order stimulus omission, the second-order stimulus was defined as the time in the schedule at which the second-order stimulus would have occurred had it been presented).

### *NEUROPHYSIOLOGY*

*Spike sorting.* Spikes were re-sorted offline in Spike 2™ (Cambridge Electronic Design Ltd., Cambridge, UK) by performing principal components analysis on 40-60 data points of every waveform in the data set. The first three principal components of each spike were then used to assign each waveform to a co-ordinate resulting in clusters of waveforms with similar features in a 3-D space. Separate clusters were then classified using the Normal Mixtures algorithm in Spike 2™ and modified to include only waveforms that were within 2.5-3 standard deviations of the centroid of that cluster. Clusters were classified as single neurons according to the following criteria: the average waveform duration was 1-2.5ms, the interspike interval histogram exhibited a refractory period, there were no signs of noise at 50Hz or its harmonics, and there were no electrical artefacts within the cluster from the rat licking the spigot or pressing the bar. Visual inspection of peri-event histograms of the neuron's firing rate relative to lick and press onset allowed for identification of rare instances when electrical

artefacts masked the true firing rate of the neuron when the rat had licked or pressed. Data from these instances were dropped from the sample. Identical neurons recorded over consecutive testing days were identified by visual inspection of the waveform shape/duration, interspike interval histogram, average firing rate, and event-related activity. In these instances neuronal data were averaged together.

*Windows for spike counts.* Using a Spike 2™ script (E. Bowman, University of St Andrews, UK) histograms, rasters and spike counts for each neuron were generated relative to time windows around the second-order stimulus onset and offset (separately for second-order stimuli that did or did not terminate the schedule), and the reinforcement signal, primary reinforcer, lick and press onsets. Since our analysis was restricted to quantifying any neural responses to second-order stimuli and primary reinforcement, we calculated the average firing rate (Hz) of each neuron within the following time windows: a 'baseline' time window was 0-0.5s prior to second-order stimulus onset, a 'second-order stimulus onset' time window was 0-0.5s following second-order stimulus onset, a 'second-order stimulus offset' time window was 0-0.5s following second-order stimulus offset, and a 'primary reinforcement' time window was the 4s of primary reinforcement delivery (see Figure 3.1).

Mixed design repeated measures ANOVA's with difference contrasts were performed on spike frequency (Hz) across second-order stimuli for each neuron over Baseline, Second-order stimulus onset and Second-order stimulus offset time windows (Main factor, *Epoch*) comparing second-order stimuli that did or did not terminate the schedule (between group factor, *Schedule position*). Neurons were classified as showing a second-order stimulus response when there was an *Epoch* main effect or an *Epoch* x *Schedule position* interaction effect ($p<=0.05$). In order to assess whether the neurons responded to the onset and/or offset of the second-order stimulus orthogonal difference contrasts independently compared firing rates within the Baseline versus Second-order stimulus onset

time windows, and the firing rates within the Second-order stimulus offset versus the average over Baseline and Second-order stimulus onset time windows. Neurons were classified as showing a reinforcement response when there was a significant difference ($p<=0.05$ within a paired samples t-test) between neural firing rate (Hz) within the Baseline and Primary reinforcement time windows across primary reinforcers for each neuron.

Details of additional analyses are presented in the appropriate figure legends and were performed using Microsoft Excel 2000™ and SPSS 10.0 for Windows™. Rasters and histograms were presented using Neuroexplorer™ (version 3; Plexon Inc., Dallas, Texas) and Spike 2™. In cases where repeated measures ANOVA was performed, the Hunyh-Feldt correction was used to decrease the effect of heterogeneity of variance.

## RESULTS

### BEHAVIOUR

As previously reported (Wilson and Bowman 2004b) we found overall response rates were significantly increased during second-order stimulus omission and decreased back to baseline levels following reinstatement of the second-order stimulus. We determined the effect of the second-order stimuli on temporal patterns of responding within the second-order schedule. We found that bar-pressing increased prior to second-order stimulus presentation and decreased for ~3s after the second-order stimulus, a pattern that was enhanced during neurophysiological recording (see Figure 3.2). Population histograms of the average lick rate relative to the onsets of the terminal second-order stimulus and the primary reinforcer for each rat per testing day shows that rats licked the reward spigot with a minimum latency of approximately 300ms after the onset of the terminal second-order stimulus and for approximately 26s following primary reinforcer onset (see Figure 3.3).

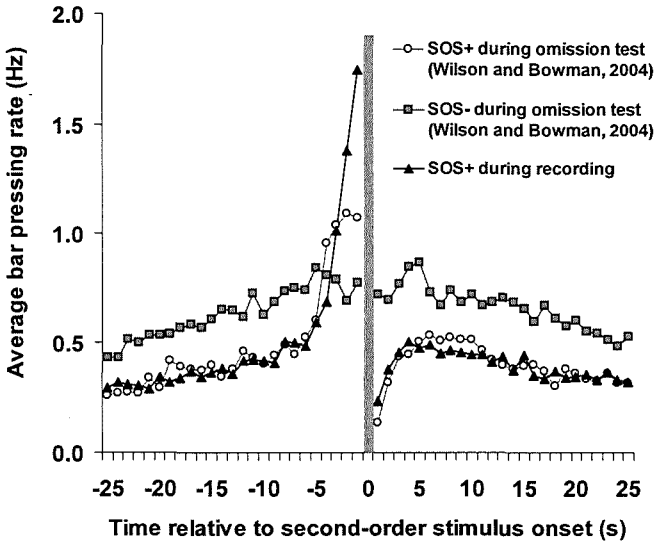



*Figure 3.2* Average bar-pressing rate (Hz) relative to second-order stimulus onset for every second-order stimulus that did not terminate the schedule, when the second-order stimulus was present ("SOS+"; open circles; n=4 rats) versus absent ("SOS-"; grey squares; n=4 rats) during the omission test and during neurophysiological recording ("SOS+"; black triangles; n=4 rats, 33 sessions). The data presented here are from the four rats from which we recorded but were also described in Wilson & Bowman, 2004. Bin size= 1 second. *N.B. Data at 0 seconds were excluded (indicated by grey bar), since every second-order stimulus was immediately preceded by at least one bar press.*

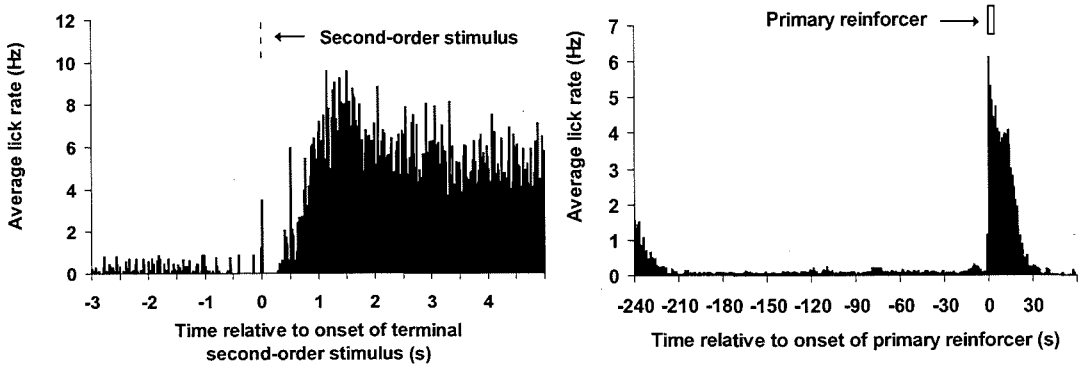



*Figure 3.3* Average lick rate (Hz) across the recording sessions where neurons were present (n=4 rats, 33 sessions) relative to the onset of the terminal second-order stimulus (*Top*; dashed line represents terminal second-order stimulus onset; bin size=10ms) and to the onset of the primary reinforcer during recording (*Bottom*; white box represents delivery of primary reinforcement; bin size=1s).

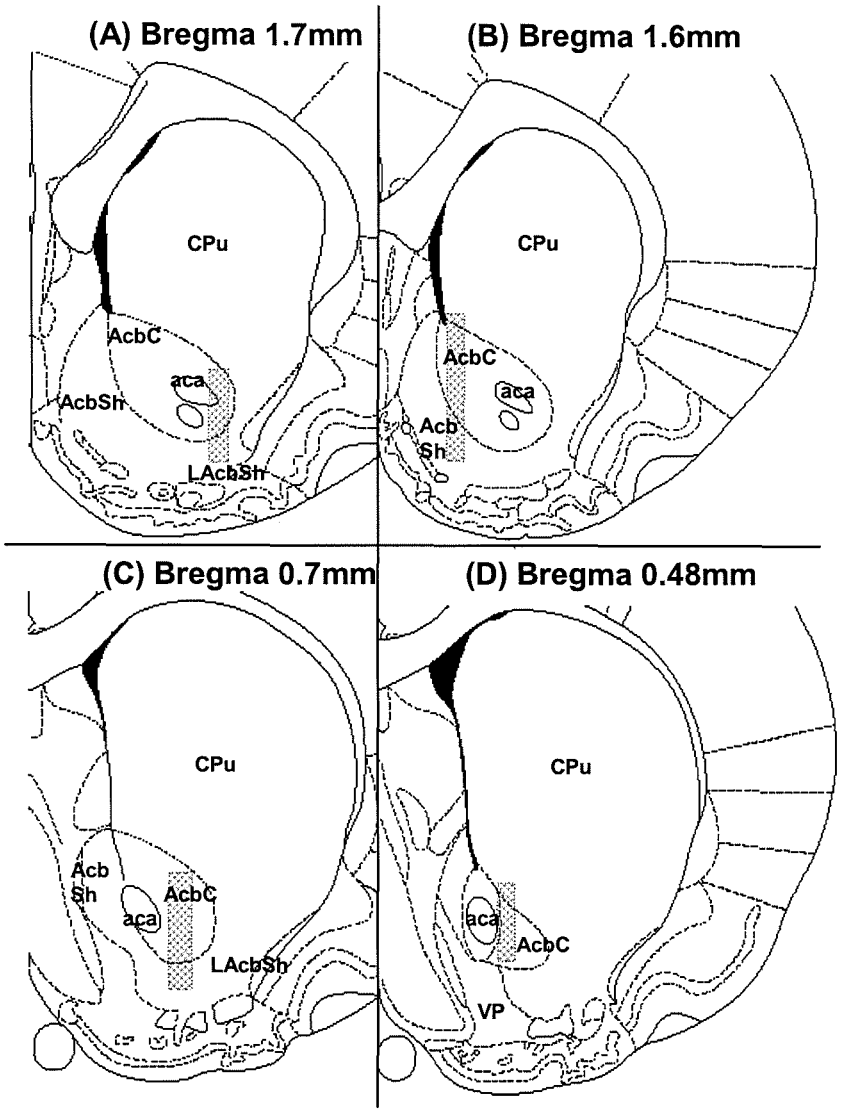



*Figure 3.4* Approximate placements of recording wires within each rat (A-D) where successful recording took place. Each overlapping diagram represents a coronal section referenced to bregma (Paxinos and Watson 1997). Grey shaded boxes represent approximate areas where recording wires were situated. All rats had wires extending into the nucleus accumbens core or shell areas over a large anterior-posterior, medial-lateral, dorsal-ventral range. In one rat (D) the wires may have extended into the border between the posterior nucleus accumbens core area and the interstital nucleus of the posterior limb of the anterior commisure and/or the ventral pallidum, accounting for approximately five neurons. Although we could not determine the precise location of each neuron there were no obvious differences between the activity of neurons between rats or within the dorsal-ventral distance traveled by the microwires within each electrode. *Abbreviations: aca*, anterior commisure, anterior part; *AcbC*, accumbens nucleus, core; *AcbSh*, accumbens nucleus, shell; *CPu*, caudate putamen (striatum); *LAcbSh*, lateral accumbens shell; *VP*, ventral pallidum. Illustration adapted from Paxinos & Watson, 1997.

*HISTOLOGY*

From the four subjects that were successfully tested neurophysiologically, electrode tracks were all within the nucleus accumbens and included portions of both the core and the shell (see Figure 3.4). However, given that we could not determine the location of each recorded neuron, we did not perform separate analyses on core *versus* shell neurons.

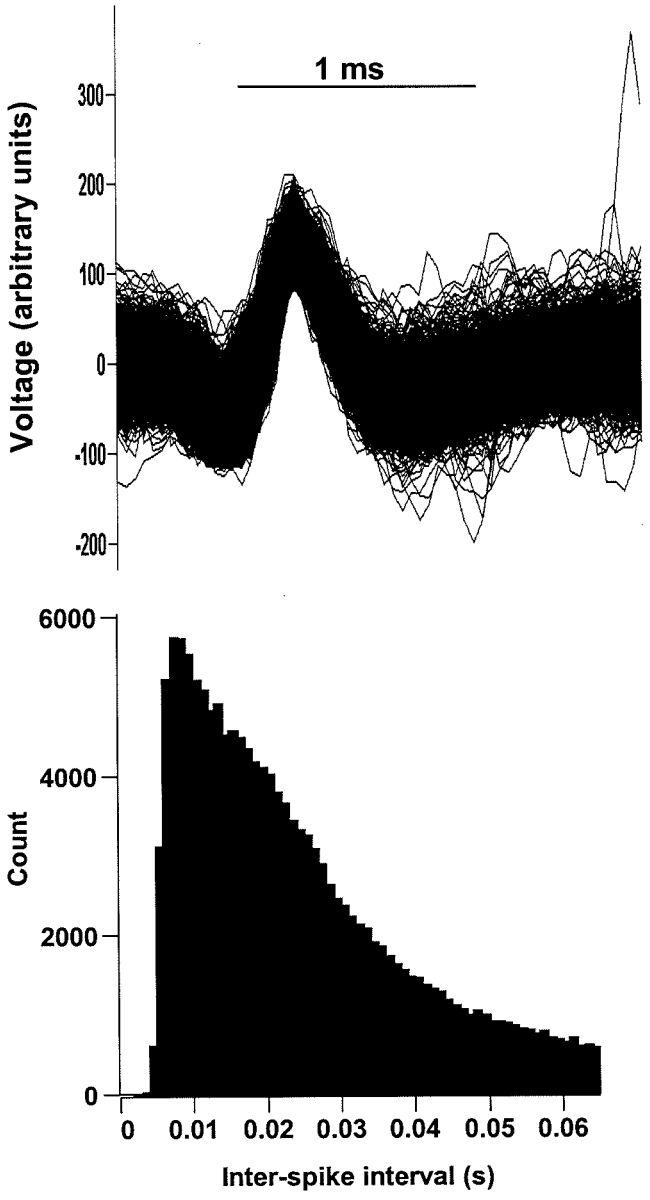


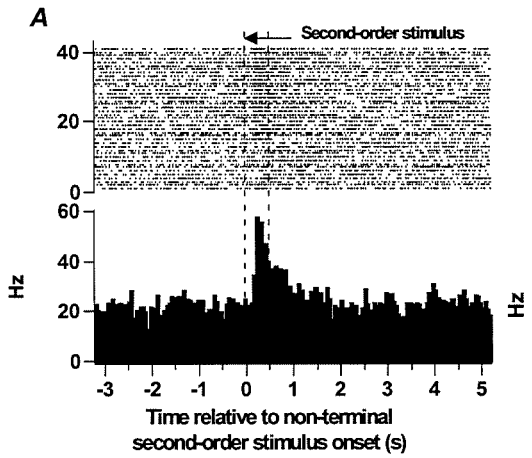

*Figure 3.5* Characteristics of an example neuron recorded whilst a rat performed on the second-order schedule of reinforcement. *Top*: Overdraw of every waveform in test session (n= 166,774 spikes). *Bottom*: Histogram of inter-spike intervals showing a clear refractory period (mode ~ 8ms; y-axis=number of action potentials; x-axis= time between consecutive spikes (s); bin size = 1ms; n= 166,774 spikes).

*RESPONSES TO SECOND-ORDER STIMULI AND PRIMARY REINFORCEMENT*

Activity from sixty-five neurons in the nucleus accumbens was collected (median firing rate 13.33 Hz (± 7.44-22.99 semi-interquartile range); see Figure 3.5 for characteristics of an example neuron) whilst rats behaved on the second-order schedule of saccharin solution reinforcement. Fifty neurons from our sample (77%) exhibited statistically significant responses to the second-order stimulus and/or the delivery of the primary reinforcer (see Table 3.1). Of these, forty-five responded to the second-order stimulus, and thirty-two responded to the primary reinforcer. Interestingly, most (84%) of the neurons that responded to the primary reinforcer also responded to the second-order stimulus. No neuron in our sample exhibited measurable motor activity (phasic activity prior to licks or bar presses). Figure 3.6 shows the characteristic response pattern of a neuron's firing relative to the onset of the second-order stimulus, reinforcement signal, primary reinforcer, bar-press and spigot-lick.

| Second-order stimulus response **only** | Reinforcement response **only** | Second-order stimulus **and** Reinforcement response | No response | Total |
|---|---|---|---|---|
| **18** (27.7%) | **5** (7.7%) | **27** (41.5%) | **15** (23.1%) | **65** |

*Table 3.1* Summary table of number (and percentage) of neurons showing a second-order stimulus and/or reinforcement response.



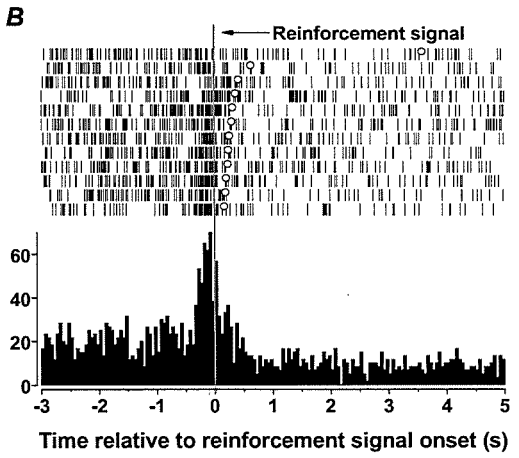
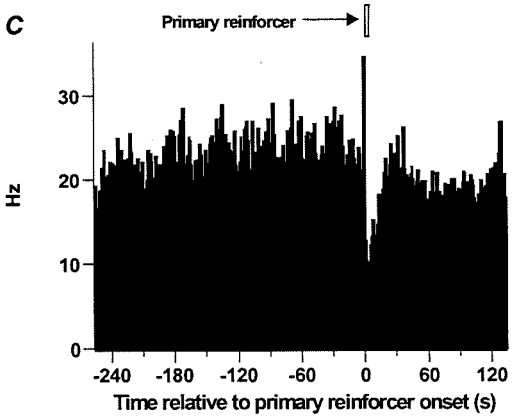
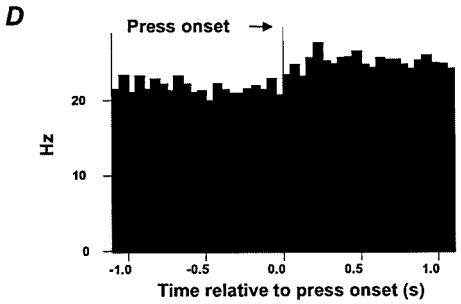
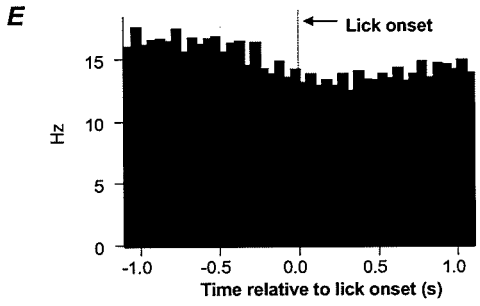




*Figure 3.6* Example from a single neuron showing phasic excitation to the second-order stimulus onset (firing rate within Second-order stimulus onset / Baseline time windows= 1.74) and inhibition during reinforcement (firing rate within Reinforcement / Baseline time windows= 0.464). Rasters and histograms of average firing rate of neuron at the onset of every second-order stimulus that did not terminate the schedule (Fig. 3.6A; raster from bottom to top shows neural firing at each trial from session start to end; dashed lines represent second-order stimulus onset and offset; bin size=50ms), the onset of every reinforcement signal (Fig. 3.6B; circles on raster represents primary reinforcer signal onset with shortest time between reinforcement signal and primary reinforcer onset from bottom to top; grey line represents reinforcement signal onset; bins size=50ms), the onset of every primary reinforcer onset (Fig. 3.6C; white box represents primary reinforcer delivery; bin size=1s), the onset of every bar press (Fig. 3.6D; dashed line represents press onset; bin size=50ms), and the onset of every lick of the reward spigot (Fig. 3.6E; dashed line represents lick onset; bin size=50ms).



*CHARACTERISTICS OF RESPONSES*

We then sought to further characterize the second-order stimulus responses. Two thirds of neurons that responded to the second-order stimulus showed no difference in their response to second-order stimuli that did or did not terminate the schedule. An equal proportion of these neurons responded to the onset as the offset of the second-order stimulus, but the majority exhibited a sustained response throughout both the onset and offset time windows (see Table 3.2). Of the remaining third of neurons that did differentiate between second-order stimuli that did or did not terminate the schedule, almost all (80%) exhibited responses at the offset of the second-order stimulus (which also was the onset of the reinforcement signal), as though these neurons responded to the second-order stimulus offset in anticipation of primary reinforcement.

| | | |
|---|---|---|
| *Neurons equally responsive between terminal and non-terminal second-order stimuli* | Stimulus onset | **9** |
| | Stimulus offset | **9** |
| | Stimulus onset and offset | **12** |
| *Neurons differentially responsive between terminal and non-terminal second-order stimuli* | Stimulus onset | **1** |
| | Stimulus offset | **12** |
| | Stimulus onset and offset | **2** |
| *No second-order stimulus response* | | **20** |
| *Total neurons sampled* | | **65** |

*Table 3.2* Summary table displays the numbers of neurons equally (*Epoch* Main effect) or differentially responsive ((*Epoch* x *Schedule position* interaction effect) to second-order stimuli that did or did not terminate the schedule as determined by ANOVA of neural firing rate (Hz) across second-order stimuli per neuron, $p<=0.05$. The latencies of these responses (second-order stimulus onset and/or offset) were determined by difference contrasts ($p<=0.05$). *N.B.* 12/15 neurons with *Epoch* x *Schedule position* interaction effect also exhibited an *Epoch* Main effect.

*VALENCE OF RESPONSES*

When we considered the valence of second-order stimulus responses we found the majority, to both the onset and offset, were excitatory (87% and 63%, respectively). In contrast, nearly all neurons that responded to the primary reinforcer were inhibited during consumption of the primary reinforcer (91%). We found a similar pattern when we analysed the valence of activity of all task-related neurons (n=50) relative to the second-order stimulus and primary reinforcer. Among the neurons in this sample, excitation was most common after the second-order stimulus onset (74%), followed by the second-order stimulus offset (56%), and was least common during primary reinforcement (28%) (see Figure 3.7). There were no differences between the proportions of neurons responding to the tone (37/55 neurons) *versus* the light (8/10 neurons) second-order stimulus.

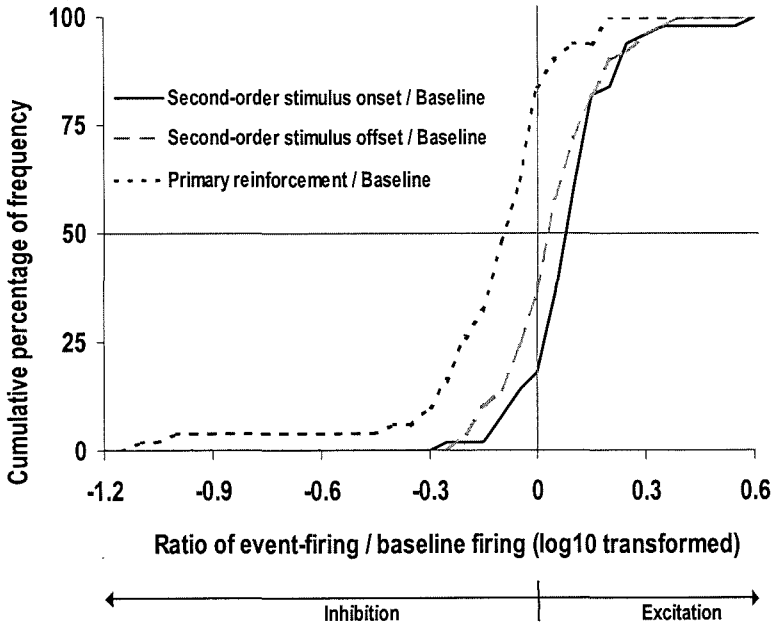



*Figure 3.7* Cumulative percentage of frequency of event-related firing / baseline firing ratios ($log_{10}$-transformed) for population of event-related neurons (n=50). Thin grey vertical line indicates $log_{10}$ (event-related firing) / $log_{10}$ (baseline firing) = 0; thin grey horizontal line indicates the $50^{th}$ percentile. Median value for $log_{10}$ (Second-order stimulus onset) / $log_{10}$ (Baseline firing) per neuron = 0.08; median value for $log_{10}$ (Second-order stimulus offset) / $log_{10}$ (Baseline firing) per neuron = 0.03; median value for $log_{10}$ (Primary reinforcement) / $log_{10}$ (Baseline firing) per neuron = -0.08.

*ANALYSIS OF INDIVIDUAL NEURONS RESPONDING TO THE SECOND-ORDER STIMULUS AND PRIMARY REINFORCER*

We next wanted to determine the pattern of activity in single neurons that responded to both the second-order stimulus and to the primary reinforcer (n=27). We compared the observed versus expected frequencies of the four possible combinations of activations to the second-order stimulus and primary reinforcer (+/+, -/-, +/-, -/+) and found a majority (78%) exhibited excitation to the second-order stimulus followed by inhibition to the primary reinforcer (+, -), as though second-order stimuli and the primary reinforcer were encoded as opposites by these neurons (see Table 3.3). However, there was no linear inverse relationship within these neurons between the magnitudes of responses to the second-order stimulus with the magnitudes of responses to the primary reinforcer (see Figure 3.8).

| | Second-order stimulus **excitation** | Second-order stimulus **inhibition** |
|---|---|---|
| Primary reinforcer **excitation** | 4 | 0 |
| Primary reinforcer **inhibition** | 21 | 2 |

*Table 3.3* Valence of responses evoked to the second-order stimulus and primary reinforcer by individual neurons that responded to both the second-order stimulus and primary reinforcement. A chi-square Goodness-of-Fit Test revealed that the probability of the four different activation patterns occurring was not equal ($\chi^2$=41.296, $p$<0.001; $H_o$: $p(row_1,column_1)=p(row_1,column_2)=p(row_2,column_1)= p(row_2,column_2)$). An additional Chi-Square Goodness-of-Fit Test showed that there was no dependence in frequencies between the rows and columns ($\chi^2$=0.376, $p$=0.540; $H_o$: $p$(cell)= $p$(row) x $p$(column)). N.B. When neurons' responded differentially between the onset and offset of the second-order stimulus, the valence of the onset response was used (n=2).

*POPULATION RESPONSES ALSO EXHIBITED A BIPHASIC PATTERN*

Having determined that individual neurons responded to second-order stimuli and/or the primary reinforcer we wanted to evaluate the neural population responses to these stimuli. The activity of our sample of neurons was similar to that found in individual neurons. Repeated measures ANOVA (with orthogonal difference contrasts) of average firing rate across neurons that responded to the second-order stimulus (n=45) revealed there was a significant 21% increase in firing rate within the second-order stimulus onset *versus* baseline time windows ($F_{(1,88)}$=41.531, $p$<0.001). There was no significant effect on the firing rates between second-order stimuli that did or did not terminate the schedule. The population of neurons that responded to the primary reinforcer (n=32) showed a 26% decrease in firing during reinforcer consumption (statistically confirmed by a paired samples t-test (t (31)= 4.913, p<0.001). Figure 3.9 shows this biphasic pattern in the form of population histograms composed of the activity of all sampled neurons (n=65). The trough of the inhibition occurred immediately at the onset of the primary reinforcer and dissipated over the subsequent 19s approximately back to baseline rate.

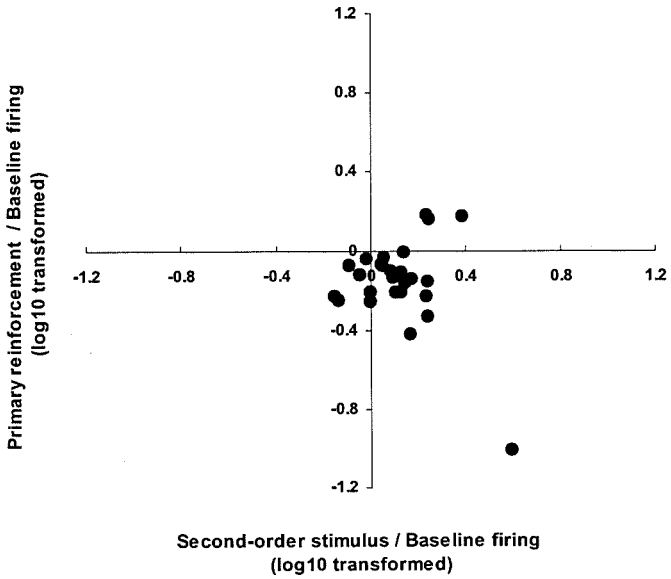



*Figure 3.8* Scatterplot of $\log_{10}$ (Primary Reinforcement) / $\log_{10}$ (Baseline firing) versus $\log_{10}$ (Second-order stimulus) / $\log_{10}$ (Baseline firing). N=27 neurons that responded to both the second-order stimulus and primary reinforcer. Spearman's rank order correlation revealed a non-significant correlation coefficient = 0.013, $p$=0.949. N.B. In cases where neurons responded to both the onset and offset of the second-order stimulus with the same valence the largest response was used (n=9). When neurons' responded differentially between the onset and offset of the second-order stimulus, the valence of the onset response was used (n=2).

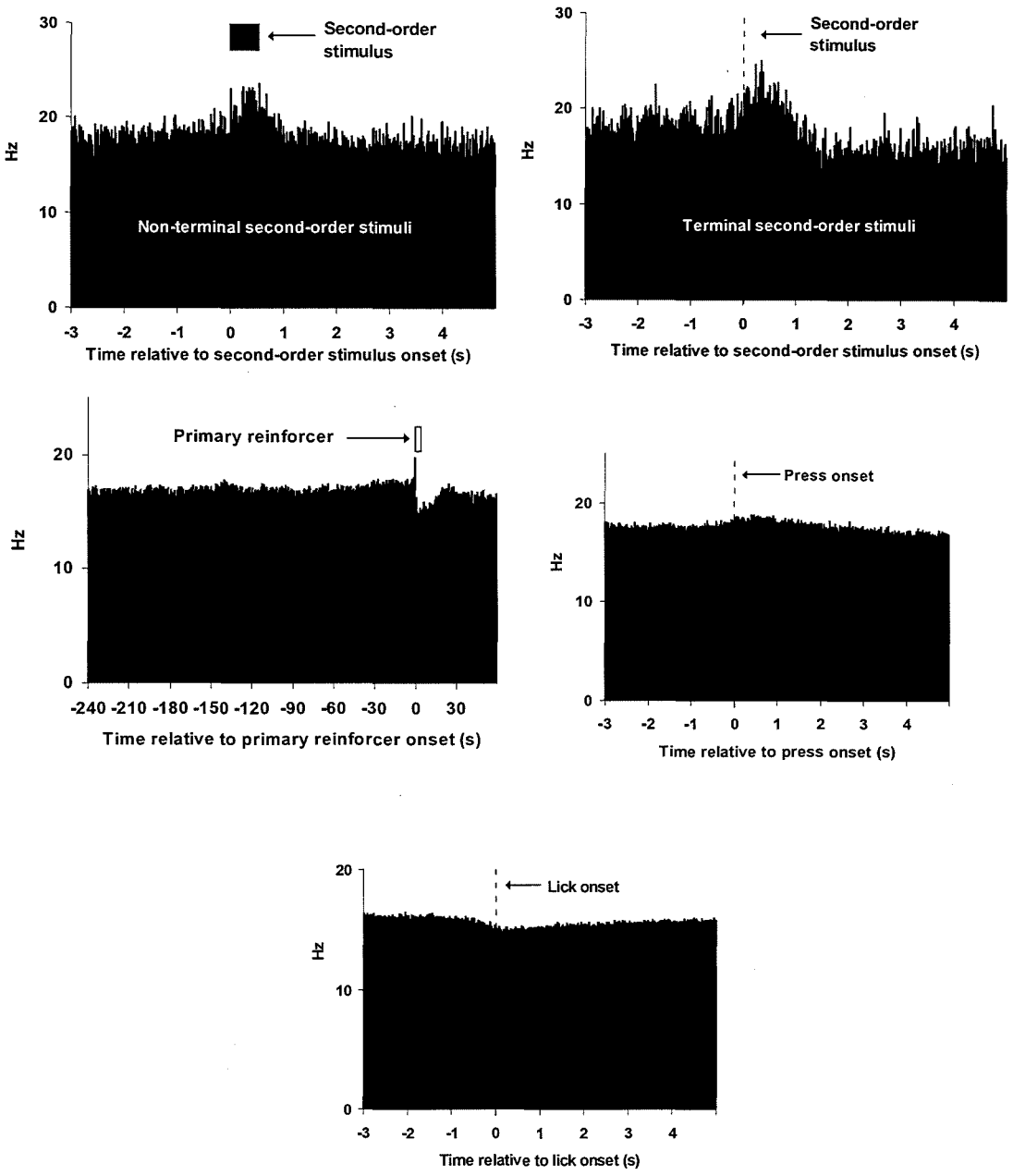



*Figure 3.9* Population histograms consisting all neurons (n=65) relative to (*Top left*) all second-order stimuli that did not terminate the schedule (black box represents the presentation of second-order stimuli that did not terminate the schedule; bin size=10ms), (*Top right*) all terminal second-order stimuli (dashed line represents the onset of the terminal second-order stimulus; bin size=10ms), (*Middle left*) all primary reinforcers (white box represents primary reinforcer onset, bin size=1s), (*Middle right*) all bar-presses (dashed line represents press onset; bin size=10ms) and (*Bottom*) all licks of the reward spigot (dashed line represents lick onset; bin size=10ms).

*EFFECT OF TEMPORAL PROXIMITY TO REINFORCEMENT ON POPULATION RESPONSES TO SECOND-ORDER STIMULI*

To determine whether neurons responding to second-order stimuli anticipated primary reinforcement, we considered the firing pattern of the neuronal population to the second-order stimulus with relation to the proximity the second-order stimulus was from the primary reinforcer (we were unable to perform this analysis on a neuron-to-neuron basis due to the low numbers of some event types within a given session). We found that the population of neurons responding to second-order stimuli (n=45) maintained the same pattern and magnitude of response to second-order stimuli, as determined by repeated measures ANOVA ($F_{(2,428)}$=20.467, $p$<0.001), irrespective of the time the second-order stimulus was presented relative to trial onset (0-1 min, 1-2min, 2-3min, 3-4mins, 4-5mins, 5-6mins), and hence relative to primary reinforcement.

*RELATIONSHIP BETWEEN POPULATION RESPONSES TO SECOND-ORDER STIMULI AND SUBSEQUENT BEHAVIOUR*

Finally, we assessed whether neurons responding to second-order stimuli triggered specific behaviours (subsequent lick, press, or neither) - again we were unable to perform this analysis on a neuron-to-neuron basis due to the low numbers of some event types within a given session. Repeated measures ANOVA with orthogonal difference contrasts revealed there was no change between responses to second-order stimuli when a lick (n=43), press (n=45), or 'no response' (n=37) was made within 5 seconds of the second-order stimulus onset.

## DISCUSSION

### SUMMARY OF RESULTS

We found that the pattern of behavioural responding within this second-order schedule during neurophysiological recording was comparable, if not more robust, than that observed previously (Wilson and Bowman 2004b). The activity of sixty-five neurons located in the nucleus accumbens was recorded while rats responded on this second-order schedule. Over three-quarters of neurons showed significant modulations in firing rate following presentation of the second-order stimulus and/or during delivery of primary reinforcement. The average response from the population of neurons showed excitation to the second-order stimulus and/or inhibition during primary reinforcement delivery. This response to the second-order stimulus remained unchanged relative to the proximity each second-order stimulus was to the primary reinforcer or relative to the type of behaviour made following it.

### NUCLEUS ACCUMBENS NEURONS WERE PREDOMINATELY EXCITED TO SECOND-ORDER STIMULI

Experimental lesions and pharmacological manipulations of the nucleus accumbens have provided indirect evidence that nucleus accumbens neurons play an important role in mediating the effects of second-order stimuli on second-order schedule responding (Di Ciano and Everitt 2001; Everitt et al. 1989; Hutcheson et al. 2001) and in attaching motivational significance to conditioned stimuli for the production of conditioned responses (Setlow et al. 2002). Although single-neuron recordings in the nucleus accumbens have not previously been made during second-order schedules, it has been reported that nucleus accumbens neurons are phasically excited to salient events occurring during reinforcer-seeking behaviour, e.g. trial onset cues, cues providing information on the type of trial, cues that trigger responding, bar presses/releases, and

reinforcer-associated cues (Bowman et al. 1996; Carelli and Ijames 2001; Chang et al. 1998; Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Nicola and Deadwyler 2000; Peoples and West 1996; Schultz et al. 1992; Shidara et al. 1998; Tremblay et al. 1998; Williams et al. 1993).

We have extended these findings by demonstrating directly that nucleus accumbens neurons were excited to second-order stimuli that partially predicted primary reinforcement. In contrast to previous neurophysiology studies, we found nucleus accumbens neurons were excited by the offset as well as the onset of reinforcer-associated stimuli. A potential explanation for this finding in our task may be that the reinforcement signal was presented immediately at the second-order stimulus offset once the rats completed the second-order schedule, which was likely to have resulted in the second-order stimulus offset becoming a salient reinforcer-predicting event. An alternative explanation is that the offset responses were in fact delayed responses bridging the gap between the second-order stimulus and the primary reinforcer. In this regard, the most common pattern of excitation lasted over both second-order stimulus onset and offset time windows. Prolonged excitation of this nature has been reported previously following the presentation of reinforcer-predictive cues extending until a trigger stimulus (signaling to the animal to make a response to earn reinforcement) or until primary reinforcement itself (Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Nicola et al. 2004b; Shidara et al. 1998; Tremblay et al. 1998).

### NUCLEUS ACCUMBENS NEURONS WERE PREDOMINATELY INHIBITED DURING PRIMARY REINFORCEMENT

We answered our first question by demonstrating that nucleus accumbens neurons, as a population, were inhibited during and following primary reinforcement. Increases in rat nucleus accumbens neuronal firing during reinforcer-seeking and decreases following the onset of primary reinforcement

have previously been reported during self-administration of cocaine (Chang et al. 1998; Nicola and Deadwyler 2000; Peoples and West 1996) and of heroin (Chang et al. 1998), and within a discriminative stimulus task for liquid sucrose reward (Nicola et al. 2004c). In contrast, some studies report phasic excitation to the primary reinforcer predominates (Bowman et al. 1996; Carelli and Deadwyler 1994; Carelli et al. 2000; Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Shidara et al. 1998; Tremblay et al. 1998). These differences are present between studies using almost identical behavioural tasks and training regimes, namely rats trained over several weeks to make one bar-press to earn primary reinforcement (Carelli and Deadwyler 1994; Carelli et al. 2000; Chang et al. 1998; Peoples and West 1996). Consequently, the differences are unlikely to be a result of the length or degree of training.

One potential explanation for the differences between these two sets of studies is the time windows used to assess neural responses to primary reinforcement. Carelli *et al.*, recording in the rat, used a fixed 0-2.5s time window relative to the single bar-press that delivered reinforcement (Carelli and Deadwyler 1994; Carelli et al. 2000). Potential excitatory neural responses during reinforcer-seeking behaviour (movement to the drinking trough/ completion of bar press response/ anticipation or expectation of reinforcement) might have been captured within this window, whilst potential consummatory/drug-taking effects occurring after this time window might have been missed. Similarly, Schultz *et al.*, recording in the monkey employed time windows relative to the onset of the reinforcement apparatus that also included a time period (55ms) prior to the arrival of the reinforcer (Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Tremblay et al. 1998). More importantly, these studies using rhesus macaques tend to use a small volume of liquid that is consumed rapidly (e.g. within ~290ms of reward apparatus onset (Hassani *et al.*, 2001, Figure 2)). This period of consumption is short compared to the window used for counting spikes in reinforcer-related responses (e.g. Hassani *et al.*, 2001, used a 2 seconds window)).

In contrast, the time windows used by researchers reporting predominately neural inhibition either began at least 1 second after the rat approached the receptacle to consume reinforcement (Chang et al. 1998; Nicola et al. 2004c), or were long enough (1min (Nicola and Deadwyler 2000); 2min (Peoples and West 1996)) to avoid neural responses to reinforcer-seeking events superceding those to the reinforcer. Here, we were able to time-stamp neural activity to the onset of consumption of the primary reinforcer as measured by the contact lickometer. The high number of task related neurons we found (77%) might reflect the tight time-locking of the window used for counting spikes to the actual consumption of the primary reinforcer. Additionally, it should be noted that some studies did not report inhibitory responses since baseline rates of neural firing were considered to be too low (Hassani et al. 2001; Hollerman et al. 1998) whilst others with similar baseline rates did (Nicola et al. 2004c; Peoples and West 1996).

## NUCLEUS ACCUMBENS NEURONS DID NOT ENCODE CONDITIONED AND PRIMARY REINFORCEMENT IN THE SAME WAY

Initially we asked whether nucleus accumbens neurons would fire in the same way to conditioned reinforcers as to primary reinforcers. In fact, the responses evoked by conditioned reinforcers and primary reinforcers were opposite in valence when the neurons were considered as a population suggesting that the neurons encoded conditioned and primary reinforcers differently. This conclusion may seem limited given that our second-order stimuli did not reinforce overall bar-pressing rates over the entire session (Wilson and Bowman 2004b). However, it has been argued previously that stimuli associated with positive outcomes can shape behaviour to form distinct temporal patterns. The generation of these patterns has been argued to constitute evidence of reinforcement (Byrd and Marr 1969; Skinner and Morse 1958; Stubbs 1971; Wilson and Bowman 2004b).

## THE POPULATION RESPONSE TO SECOND-ORDER STIMULI DID NOT VARY WITH REINFORCEMENT PROXIMITY

In answer to our third specific question, excitation following second-order stimuli was not differential depending on their proximity to primary reinforcement. It is possible that the association between the second-order stimulus and primary reinforcement was weaker than expected since it has been demonstrated previously that nucleus accumbens neurons do encode proximity to reinforcement in some circumstances (Bowman et al. 1996; Shidara et al. 1998). Consequently, temporal information used to complete the fixed interval component of the second-order schedule is likely have been derived internally. We were unable to detect any temporal information in our sample, since firing rates remained relatively constant over the four-minute fixed interval (see population histogram 12C).

## CONTRIBUTION BY NEURONS TO BEHAVIOUR

Our sample of nucleus accumbens neurons, considered as a whole, did not predict subsequent behaviour (namely pressing or licking), yet others have found that nucleus accumbens neurons exhibit differential responses to reinforcer-predictive stimuli gated by the presence of absence of a subsequent behavioural response (Nicola et al. 2004b; Setlow et al. 2003). However, Setlow *et al.* noted these responses were found within a subpopulation of their neuronal sample that had relatively low 'baseline' firing rates (<3Hz), and not in those with relatively high rates (>5Hz). Given that our neurons had relatively high 'baseline' firing rates our sample seems comparable to the latter subgroup reported by Setlow *et al.* (2003). Setlow *et al.* also reported that slower firing neurons were usually sampled when rats had a high level of task performance (discriminative responding with few errors). It is plausible that we did not encounter these types of responses because our rats had not fully learnt the circumstances under which conditioned reinforcers accurately predicted primary reinforcement.

We conclude that the responses of neurons in our sample did not respond equivalently to conditioned and primary reinforcers, or trigger signals for subsequent motor responses. Instead, the function of excitatory responses to second-order stimuli may have been to track motivationally salient events during reinforcer-seeking. It is possible that these responses could indirectly influence patterns of behaviour through extensive upward-spiraling reciprocal connections with midbrain dopamine neurons that ascend through motivational (ventromedial), cognitive (central) and motor (dorsolateral) areas of the striatum (Haber et al. 2000; Robbins and Everitt 2002). Similarly, the function of inhibitory responses to primary reinforcement may also have been to influence patterns of behaviour signaling that the goal had been achieved, thereby allowing another behavioural sequence to be initiated. Given that many neurons (23/65) responded to either the second-order stimulus or to the primary reinforcer, whilst other neurons (21/65) responded to both, it is probable that these responses arose from independent but partially overlapping mechanisms within the nucleus accumbens. Further evidence supporting this conclusion is that the magnitudes of responses to second-order stimuli and primary reinforcers, in neurons responsive to both, were not correlated. Since reinforcer-seeking can equate with "wanting" and reinforcer consumption contributes to "liking" of primary reinforcement our findings may extend previous work proposing that "wanting" and "liking" psychological processes are modulated independently within the nucleus accumbens by dopaminergic and opioid mechanisms, respectively (Berridge and Robinson 1998; Ikemoto and Panksepp 1999; Kelley et al. 2002; Kelley and Berridge 2002; Robbins and Everitt 1996; Salamone and Correa 2002). Future studies will be required to confirm this hypothesis.

## CHAPTER 4

# RAT NUCLEUS ACCUMBENS NEURONS PREDOMINANTLY RESPOND TO THE OUTCOME-RELATED PROPERTIES OF CONDITIONED STIMULI RATHER THAN THEIR BEHAVIOURAL-SWITCHING PROPERTIES

The work presented in this chapter is currently in press for publication (Wilson and Bowman, 2005, Journal of Neurophysiology, in press).

**ABSTRACT**

It has been proposed that nucleus accumbens neurons respond to outcome (reward and punishment) and outcome-predictive information. Alternatively, it has been suggested that these neurons respond to salient stimuli, regardless of their outcome-predictive properties, to facilitate a switch in ongoing behaviour. We recorded the activity of 82 single nucleus accumbens neurons in thirsty rats responding within a modified go/no-go task. The task design allowed us to analyse whether neurons responded to conditioned stimuli that predicted rewarding (saccharin) or aversive (quinine) outcomes, and whether the neural responses correlated with behavioural switching. Approximately one third (28/82) of nucleus accumbens neurons exhibited 35 responses to conditioned stimuli. Over two-thirds of these responses encoded the nature of the upcoming rewarding (19/35) or aversive (5/35) outcome. No response was selective solely for the switching of the rat's behaviour, although the activity of approximately one third of responses (11/35) predicted the upcoming outcome and was correlated with the presence or absence of a subsequent behavioural switch. Our data suggest a primary functional role for the nucleus accumbens in encoding outcome-predicting information and a more limited role in behavioural switching.

## INTRODUCTION

It has been hypothesized that the nucleus accumbens processes outcome (reward and punishment), and outcome-predicting information (Berridge and Robinson 1998; Ikemoto and Panksepp 1999; Parkinson et al. 2000a; Robbins and Everitt 2002, 1996; Salamone and Correa 2002; Wise 1982). Findings from neurophysiological studies seem consistent with this 'outcome-prediction' hypothesis, with demonstrations of single nucleus accumbens neuronal responses to outcome delivery and to a diverse range of actions and stimuli that predict an upcoming outcome (Bowman et al. 1996; Carelli and Ijames 2001; Carelli et al. 2000; Chang et al. 1998; Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Nicola et al. 2004b; Setlow et al. 2003; Shidara et al. 1998; Wilson and Bowman 2004a).

Alternatively, it has been hypothesized that nucleus accumbens neurons respond to salient stimuli, irrespective of their outcome-predictive properties, to facilitate a subsequent behavioural switch by the organism (Bakshi and Kelley 1991a, b; Cools 1980; Evenden and Carli 1985; Evenden and Robbins 1983a, b; Horvitz 2002; Oades 1985; Reading and Dunnett 1991; Reading et al. 1991; Redgrave et al. 1999a, b; Robbins and Koob 1980; Robbins and Sahakian 1983; van den Bos and Cools 2003). Thus, it is possible that single nucleus accumbens neural responses to outcome or outcome-predictive stimuli are in fact responses to salient stimuli to cause a switch in the organism's subsequent behavioural sequence.

We aimed to test whether nucleus accumbens neurons respond to process outcome prediction and/or a switch in the rat's subsequent behaviour by recording the activity from single neurons in the nucleus accumbens of thirsty rats responding within a modified go/no-go task. Rats were trained to make responses (bar-presses or spigot-licks) that were followed by the presentation of conditioned stimuli signaling the availability of either a rewarding (sweet liquid) or

aversive outcome (bitter liquid). Rats could either make a 'go' response to trigger the outcome delivery or they could withhold their response to avoid outcome delivery. Depending on the initial response (pressing *versus* licking) and the subsequent 'go/no-go' response, there was either a switch or 'no-switch' in the rat's behaviour following reward/aversive-predictive conditioned stimuli. This design allowed us to answer the following questions: (1) Do neurons respond primarily in anticipation of the upcoming outcome, to the switching of the animal's behaviour irrespective of the upcoming outcome, or to a combination of outcome-prediction and switching information? (2) Is the valence of neural response differential between upcoming outcome types and/or to the presence *versus* absence of a behavioural switch?

## METHODS

### SUBJECTS

Eleven Listar Hooded adult male rats (Harlan UK), weighing 411g (± 59g, 95% CI) when training began, were housed in quadruplets on a light 12h: dark 12h light cycle. During experimental procedures rats were placed on a regime of restricted water access with free access to water available from 4-5PM each weekday and from Friday 4PM until Sunday afternoon. The rats' body weights were not allowed to dip below 85% of their free-drinking weight. Following surgery rats were housed singly. The "Handbook of Laboratory Animal Management and Welfare" (Wolfensohn and Lloyd 1998) was followed and all procedures conformed to the United Kingdom 1986 Animals (Scientific Procedures) Act.

### APPARATUS

#### *BEHAVIOUR*

Rats were trained in sound-attenuated testing chambers (34cm x 29cm x 25cm; Med Associates Inc., St Albans, VT) fitted with video cameras (Santec smart vision, model VCA 5156, Sanyo Video Vertrieb GmbH Co., Ahrensburg, Germany). Located on the left wall of each chamber were a retractable lever (left side), drinking spigot (centre), houselight (top centre) and piezoelectric buzzer (behind spigot; model EW-223A, Med Associates Inc.). Liquids were delivered through the drinking spigot at a rate of 0.05ml/sec by two computer controlled syringe pumps (model PHM - 100, Med Associates Inc.) through 50ml glass syringes (Rocket, London) with stainless steel plungers to ensure repeatable flow rates. One of these syringes dispensed 0.25% w/v sodium saccharin solution while the other dispensed 0.2% w/v quinine hydrochloride solution. These solutions were delivered through separate lines of Teflon tubing to avoid mixing. Solutions were delivered at precise times with reliable flow rates since the stiff

syringes, plungers and tubing prevented pressure waves produced by the pumps from being attenuated.

*NEUROPHYSIOLOGY*

Electrode arrays containing a movable bundle of four 50µm stainless steel microwires coated in Teflon (tip impedance 0.5-1.5MΩ) were used. Differential activity from two pairs of wires was amplified, filtered and then processed by a CED 1401$^{TM}$ data acquisition system (Cambridge Electronic Design, Cambridge, UK). Although the rate at which data were sampled on the CED$^{TM}$ system was 20kHz, the resolution for timestamping behavioural events was limited to that of the MED-PC$^{TM}$ system (2ms). All neurophysiology apparatus, surgical, histological and spike sorting techniques were identical to those described previously in Wilson and Bowman (2004).

PROCEDURES

During the development of the behavioural task, the first group of rats (n=7) received slightly different procedures to those described below, namely initially lower concentrations of quinine, different lengths of timeouts, different amounts of training per stage. However, the final testing stage was identical between the two groups. Rats were advanced to each stage in the training when response levels reached an asymptote. In cases in which responding ceased at a given stage, rats were either moved back to an earlier stage for re-training, or advanced to the subsequent stage when appropriate.

*TRAINING STAGE 1: LICKING RESPONSES TO EARN REWARDING AND AVERSIVE OUTCOMES*

*(A)* Rats were initially trained for a single 30-minute session using the following procedure: when the animal first licked the drinking spigot there was a variable delay of 0.1, 0.2, 0.4, or 0.8s. Rats were subsequently presented with the reward-predictive conditioned stimulus, lasting 0.5s. The rats were divided into two

groups of conditioned stimulus modality with one group (n=7) receiving a tone reward-predictive conditioned stimulus using the piezoelectric buzzer, and the other group (n=4) receiving a light reward-predictive conditioned stimulus using the houselight. After the first presentation of the conditioned stimulus (*conditioned stimulus-1*) there was a 1s-delay, followed by a second presentation of the same reward-predictive conditioned stimulus (*conditioned stimulus-2*). The double presentation of the same conditioned stimulus gave the rats a time window to prepare their subsequent 'go/no-go' response. If a lick was made within the next 2s the animal received 0.1ml saccharin solution reward lasting two seconds, along with the continued presentation of conditioned stimulus-2. At the end of the lick bout (defined as an inter-lick interval >= 300ms) there was a period of 4 seconds, unsignalled to the rat, prior to the beginning of the next trial. If no lick was made within 2s of conditioned stimulus-2 then an error was recorded, the conditioned stimulus turned off, and there was timeout period of 2.5 seconds, unsignalled to the rat, before starting the next trial.

*(B)* In a second 30-minute session, the rats were required to lick on a variable ratio-3 schedule (random selection of 1-5 licks per trial) to initiate the processes outlined above for obtaining saccharin reward.

*(C)* Rats were then trained over 2-5 daily 30-minute sessions, as outlined above except the reward-predictive conditioned stimulus was replaced by an aversive-predictive conditioned stimulus (a tone or a light, but not the same as the reward-predictive stimulus) on 1 out of 3 trials (pseudo-randomly determined on a trial-to-trial basis). On these trials, aversive quinine solution (0.1ml) was delivered as the outcome.

*TRAINING STAGE 2: PRESSING RESPONSES TO EARN REWARDING AND AVERSIVE OUTCOMES*

*(A)* The next stage replaced the operant licking responses with bar-pressing responses. Over 7 daily 30-minute sessions rats learned to perform one bar-

press in order to receive reward-predictive conditioned stimuli (no aversive trials at this stage) and then reward (initially the 2s-time window that allowed the rat to lick for reward following the onset of the conditioned stimulus-2, was increased to 10s).

*(B)* One 30-minute session was then given where rats were required to press on a variable ratio-3 schedule on each trial instead of a single press.

*(C)* Finally, during two 30-minute sessions rats pressed on a variable ratio-3 schedule for either saccharin, or quinine outcomes (see stage 1C).

*TRAINING STAGE 3: PRESSING AND LICKING RESPONSES TO EARN REWARDING AND AVERSIVE OUTCOMES*

*(A)* Rats were trained for approximately 3 weeks to press or lick on a variable ratio-3 schedule for saccharin or quinine outcomes. The operant response required by the rat was the same for ten consecutive trials, which constituted a block of trials. The first block of trials was randomly assigned as pressing or licking. The block type was then sequentially alternated and signaled to the rat by protrusion or withdrawal of the bar, respectively. Figure 4.1 illustrates this final testing stage of the modified go/no-go task.

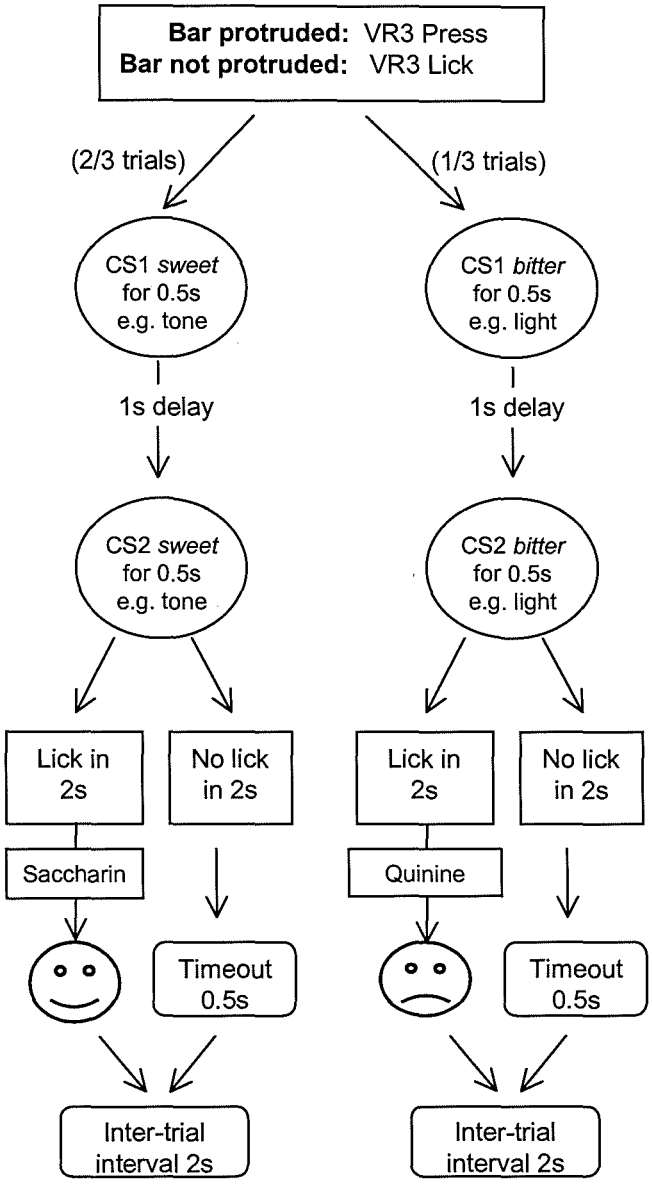
*SURGERY*

Following behavioural training, an electrode array was permanently implanted onto the skull of each rat with the electrode targeted stereotaxically at the nucleus accumbens (+1.7mm anterior and +1.5mm lateral from bregma; -6.0mm ventral to skull surface).

*NEUROPHYSIOLOGICAL RECORDING*

Rats were given 5-7 days to recover from surgery. We then recorded successfully from 8 rats whilst they behaved during the modified go/no-go task. Neural recording lasted approximately four weeks.

*Figure 4.1* Schematic representation of behavioural procedures within a given trial during the testing stage of the modified go/no-go task. *From top:* Rats worked through alternating blocks of trials (ten trials/block) requiring operant responding on a variable ratio-3 of licking or pressing. Following responding, the reward-predictive conditioned stimulus (CS sweet; on average 2/3 trials) or aversive-predictive conditioned stimulus (CS bitter; on average 1/3 trials) was presented for 0.5s. Following a 1s delay a second presentation of the stimulus was made. If the rat made a 'go' response (lick at the spigot) within 2s of this stimulus then rewarding saccharin solution or aversive quinine solution was delivered, respectively. The trial stopped when the lick bout following the offset of outcome delivery ended (defined as an inter-lick interval > 300ms), and an inter-trial interval of 2s was started (unsignaled to the rat). If the rat made a 'no-go' response (no lick at the spigot) within 2s of the conditioned stimulus there was a timeout of 2.5s. The timeouts ensured go trials and no-go trials were approximately equivalent in length.




*HISTOLOGY*

Following neurophysiological recording rats were killed by overdose with 0.7ml Dolethal™ (200 mg/l pentobarbitone sodium BP; Univet Ltd., Oxford, UK) and perfused intracardially with 0.1% phosphate buffer saline followed by a fixative (4% paraformaldehyde in 0.1M phosphate buffer). The paths of electrode tracts

were mapped onto standardised sections of the brain (Paxinos and Watson 1997).

## DATA ANALYSIS

### *BEHAVIOUR*

We restricted our behavioural analysis to the testing sessions within which activity from classified nucleus accumbens neurons was sampled (n=41 sessions). We independently performed repeated-measures ANOVA on the average percentage of trials within which rats made 'go' responses, and the average lick rates during outcome delivery, respectively, over two within-subject factors, *Operant response type* (licking *versus* pressing) and *Outcome type* (rewarding *versus* aversive).

### *NEUROPHYSIOLOGY*

*Spike sorting.* Spikes were re-sorted offline in Spike2$^{TM}$ by performing principal components analysis on every waveform in the data set. When identical neurons were recorded over consecutive testing days (as identified by visual inspection of the waveform shape/duration, interspike interval histogram, average firing rate, and event-related activity) we only used data from the session within which the rat responded maximally.

*Windows for spike counts.* Using Spike2$^{TM}$, we constructed histograms, rasters and spike counts for each neuron and for the average response of the population of neurons. These were generated relative to time windows around both conditioned stimulus-1 and 2, the quinine and saccharin delivery, and the lick and press onsets. There appeared to be a consistent pattern of neural responses that occurred phasically after presentations of conditioned stimulus-1 and 2, as well as throughout reward delivery. We restricted our analysis to quantify responses only to the presentation of conditioned stimulus-1, and to the aversive and rewarding outcomes, since neurons showing visible responses to the conditioned

stimulus-2 also showed responses at conditioned stimulus-1, which were usually of greater magnitude. We calculated the average firing rate (Hz) of each neuron within three time windows after the onset of conditioned stimulus-1 (0-100ms ('baseline'), 100-200ms, and 200-300ms (to capture any late responses)). We also compared the average firing rate around a baseline window around the trial onset (-1s to 1s trial onset) to firing during the 2s of outcome delivery.

*Assignment of trial types.* We were able to analyse the effect of outcome-prediction on neural responses since ~66% of trials per session had conditioned stimuli predicting a rewarding outcome ('CS1 sweet', Figure 4.1) and ~33% conditioned stimuli predicting an aversive outcome ('CS1 bitter', Figure 4.1). Additionally, we were able to examine the effects of the neural responses on rat's subsequent switching behaviour, since in each trial the rats either made a subsequent behavioural switch *or* no-switch (see Figure 4.2). Trials were defined as containing a behavioural switch either when the rat switched from operant bar-pressing to spigot-licking following conditioned stimulus presentation (Figure 4.2A) or when there was a switch from operant spigot-licking to avoidance of spigot-licking (Figure 4.2B). Conversely, trials were defined as containing no behavioural switch when operant spigot-licking was continued throughout conditioned stimulus presentation. (Figure 4.2C). It should be noted that one trial type was excluded from analysis (Figure 4.2D) since it was unclear whether the rat made a behavioural switch.

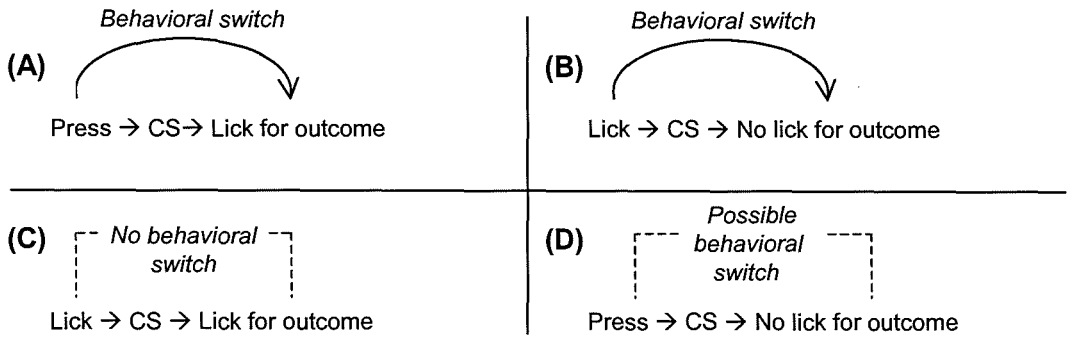



*Figure 4.2* Schematic representation of the trial types classified within our operational definition of behavioural switching. *(A)* Following a variable ratio-3 pressing schedule, the rat switched its behaviour from bar-pressing to spigot-licking following presentation of most reward-predictive and some aversive-predictive conditioned stimuli (CS), respectively. *(B)* Following a variable ratio-3 licking schedule, the rat switched its behaviour from spigot-licking approach behaviour to avoidance of the spigot following presentation of few reward-predictive and most aversive-predictive conditioned stimuli, respectively. *(C)* Following a variable ratio-3 licking schedule, the rat maintained licking (and thus made no switch in behaviour) to gain outcome delivery subsequent to most reward-predictive and a substantial minority of aversive-predictive conditioned stimuli. *(D)* Following a variable ratio-3 pressing schedule, the rat did not switch behaviour to lick the spigot for outcome delivery. However, since it was hard to define the onset and offset of bar-pressing behaviour (often the rat's paw remained on the bar without fully depressing it) we could not determine whether rats switched to another behaviour or made no switch in behaviour and continued to bar-press. Thus, these trials were dropped from the analysis.

*Classification of response type.* Mixed design repeated-measures ANOVA's with pairwise comparisons were performed on spike frequency (Hz) across each trial per neuron over the three time windows around the presentation of conditioned stimulus-1 (repeated-measures factor, *Epoch*) comparing conditioned stimuli that predicted aversive *versus* rewarding outcomes (between-group factor, *Outcome type*) and conditioned stimuli that caused the rat to make a switch *versus* no-switch response (between-group factor, *Switching type*). Neurons were classified as exhibiting an outcome-predicting response (reward outcome-predicting or aversive outcome-predicting) to the conditioned stimulus when there was a

significant *Epoch*Outcome type* interaction (*p<=0.05)* and a significant pairwise comparison (*p<=0.05)* between two of the three epoch time windows (0-100ms *versus* 100-200ms, 0-100ms *versus* 200-300ms, 100-200ms *versus* 200-300ms) following conditioned stimuli predictive of the rewarding ('CS1 sweet', Figure 4.1) or aversive ('CS1 bitter', Figure 4.1) outcome. Neurons were classified as exhibiting a switching response (switch or no switch) to the conditioned stimulus when there was a significant *Epoch*Switching type* interaction (*p<=0.05)* and a significant pairwise comparison (*p<=0.05)* between two of the three epoch time windows following conditioned stimuli that caused the rat to switch (Figure 4.2A and 4.2B) or 'not-switch' (Figure 4.2C). Neurons were classified as exhibiting an outcome-switching response (reward-switch, reward-no switch, aversive-switch, aversive-no switch) to the conditioned stimulus when there was a significant *Epoch*Outcome type*Switching type* interaction (*p<=0.05)* and a significant pairwise comparison (*p<=0.05)* between two of the three epoch time windows following conditioned stimuli that caused the rat to switch or 'not-switch' and were predictive of one type of outcome. It was possible that a neuron could satisfy more than one of these criteria and be classified as having more than one response. When repeated-measures ANOVA was performed, the Hunyh-Feldt correction was used to decrease the effect of heterogeneity of variance. When multiple pairwise comparisons were made, the Sidak test was performed to adjust for multiple comparisons.

Mixed design repeated-measures ANOVA's with pairwise comparisons were also performed on spike frequency (Hz) across each trial per neuron over a baseline (-1s to +1s trial onset) and outcome delivery time windows (Repeated-measures factor, *Epoch*) comparing aversive *versus* rewarding outcomes (between group factor *Outcome type*). This baseline time window was employed to allow us to keep the baseline and response time windows of equal length. Neurons were classified as exhibiting an outcome response if there was a significant *Epoch*Outcome type* interaction effect (*p<=0.05*) and a significant pairwise comparison between aversive and rewarding (*p<=0.05)* outcome types. Details of

additional analyses are presented in the appropriate figure legends and were performed using Microsoft Excel 2000™ and SPSS 10.0 for Windows™. Rasters and histograms were presented using Spike 2™.

BEHAVIOUR

As shown in Figure 4.3, rats found saccharin delivery rewarding, as indicated by the high lick rates, and quinine delivery aversive, as indicated by the very low lick rates (it has also been demonstrated previously that oral injections of quinine in rats causes aversive facial reactions (Grill and Norgren 1978)). We wanted rats to learn the associations between conditioned stimuli and the upcoming outcome. This appears to have been the case since rats changed their behavioural response following presentation of conditioned stimuli to avoid quinine and consume saccharin (see Figure 4.4). Finally, we sought to identify different trial types on the basis of switching/not-switching of the rat's behavioural response following conditioned stimulus presentation. Analysis of the population licking responses indicates that there was continued licking from pre- to post-conditioned stimulus presentation in 'no-switch' trials (see Figure 4.5A) and a switch from licking to not-licking (see Figure 4.5B) or pressing to licking (see Figure 4.5C) in 'switch' trials.

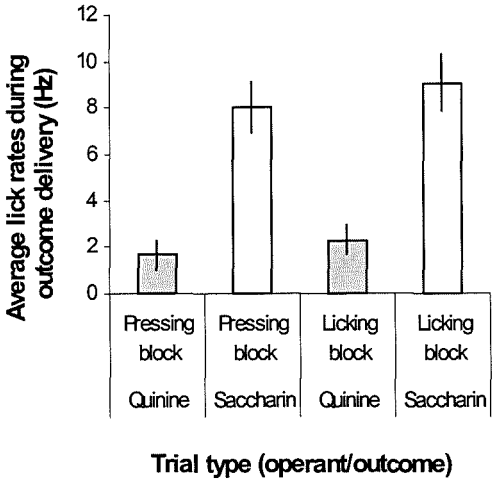
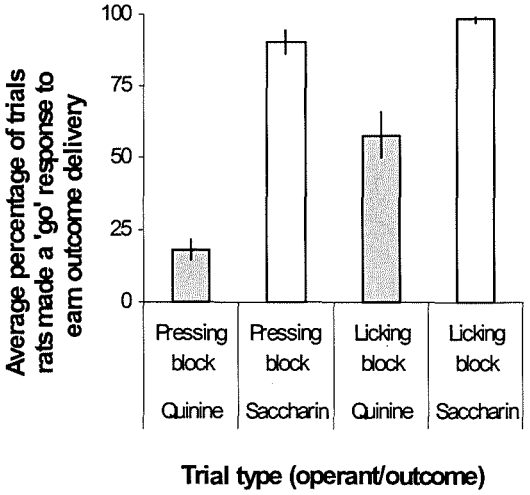



*Figure 4.3* Average licking rates (Hz; ±95% CI) during quinine and saccharin primary reinforcement following pressing and licking operant responses for rats (n=41 sessions from 7 rats) during successful recording from nucleus accumbens neurons (n=82). Repeated-measures ANOVA revealed rats licked significantly faster to delivery of saccharin *versus* quinine ($F_{(1,35)}$=207.75, $p<0.001$) and at the same rate between blocks of trials requiring pressing *versus* licking operant responses ($F_{(1,35)}$=0.122, $p=0.728$).

*Figure 4.4* Average percentage of trials (±95% CI) rats (n=41 sessions from 7 rats) made 'go' responses to earn quinine and saccharin delivery following pressing and licking operant responses during successful recording from nucleus accumbens neurons (n=82). Rats made fewer 'go' responses for quinine delivery under both responding conditions and fewer 'go' responses following pressing responses over both outcome types. Repeated-measures ANOVA revealed this was a significant *Outcome type*Operant response type* interaction ($F_{(1,40)}$=26.273, *p<0.001*).




NEUROPHYSIOLOGY

*HISTOLOGY*

From the eight subjects that were successfully tested neurophysiologically, seven had electrode tracks within the nucleus accumbens that included portions of both the core and the shell over a large anterior-posterior range (see Figure 4.6). However, given that we could not determine the location of each recorded neuron, we did not perform separate analyses on core *versus* shell nucleus accumbens neurons.

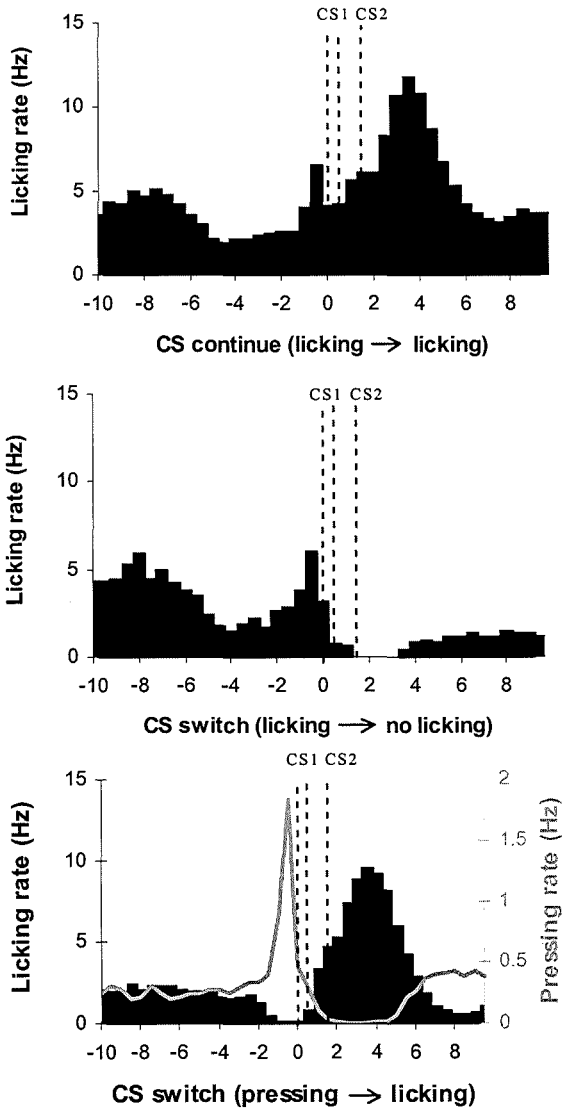



*Figure 4.5* Average licking rates (Hz) by rats (n=41 sessions from 7 rats) relative to conditioned stimulus-1 (CS) presentation during trials that were classified as (*top*) no-switch since operant licking was continued following presentation of conditioned stimulus-1, (*middle*) 'switch' trials within which operant licking was aborted following presentation of conditioned stimulus-1, and (*bottom*) 'switch' trials within which rats switched from operant bar-pressing to spigot-licking following presentation of conditioned stimulus-1. Bin size=500ms.

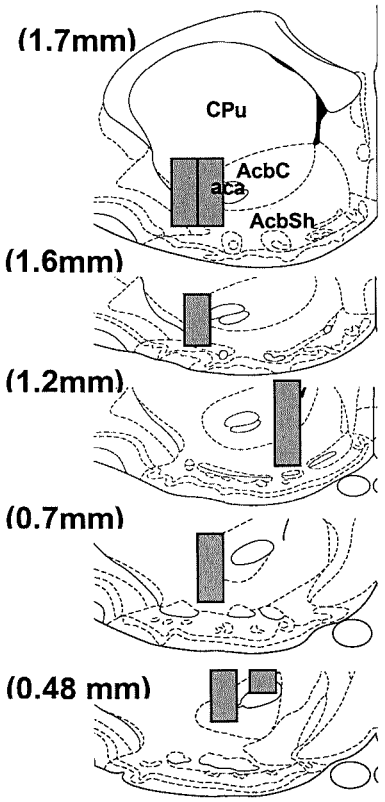



*Figure 4.6* Approximate placements of recording wires within each rat where successful recording took place. Each overlapping diagram represents a coronal section referenced to bregma (Paxinos and Watson 1997). Grey shaded boxes represent approximate areas where recording wires were situated. All rats had wires extending into the nucleus accumbens core or shell areas over a large anterior-posterior, medial-lateral, dorsal-ventral range. Although we could not determine the precise location of each neuron there were no obvious differences between the activity of neurons among rats or within the dorsal-ventral distance traveled by the microwires within each electrode. *Abbreviations: aca*, anterior commisure, anterior part; *AcbC*, accumbens nucleus, core; *AcbSh*, accumbens nucleus, shell; *CPu*, caudatoputamen (striatum). Illustration adapted from Paxinos & Watson, 1997.

*NEURONS PREDOMINANTLY RESPONDED TO OUTCOME-PREDICTION RATHER THAN BEHAVIOURAL SWITCHING INFORMATION*

We recorded from 82 neurons within the nucleus accumbens (median firing rate 6.57Hz (2.50-13.96 semi-interquartile range); see Figure 4.7 for characteristics of an example neuron) whilst rats behaved during the modified go/no-go task. Our first goal was to determine whether neurons, in general, responded as if in anticipation of the upcoming outcome or in switching the animal's behaviour. We attempted to do this primarily through analysis of the neural responses to conditioned stimuli. We found that 28/82 (34%) neurons were responsive to conditioned stimuli producing 35 event-related responses (see Table 4.1). Most of these neurons (20/28) produced responses that were classified as outcome-predicting (see Figure 4.8 for a typical response pattern). It should be noted that there were no differences in neural responses in rats trained with the light as the reward-predictive conditioned stimulus (n=3; e.g. see Figures 4.8 and 4.9), *versus* rats trained with the tone as the reward-predictive stimulus (n=4; e.g. see Figure 4.10).

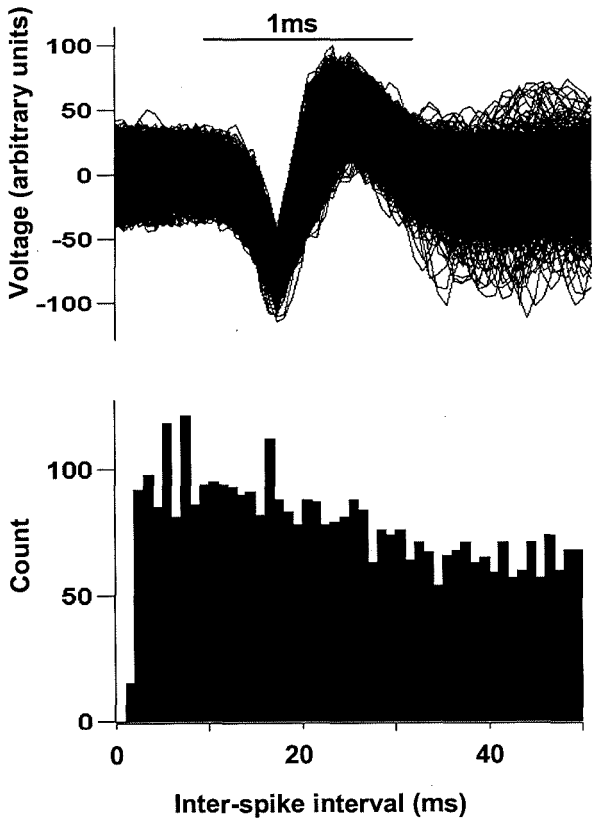



*Figure 4.7* Characteristics of an example neuron recorded whilst a rat performed the modified go/no-go task. *Top:* Superimposition of every waveform in test session (n= 13,289 spikes). *Bottom:* Histogram of inter-spike intervals showing a refractory period (mode ~ 7ms; y-axis=number of action potentials; x-axis= time between consecutive spikes (s); bin size = 1ms; n= 13,289 spikes).

| Outcome-predicting responses | | Switching responses | | Outcome-switching responses | |
|---|---|---|---|---|---|
| Reward | *19* | Switch | *0* | Reward-switch | *4* |
| Aversive | *5* | No switch | *0* | Reward-no switch | *4* |
| | | | | Aversive-switch | *1* |
| | | | | Aversive-no switch | *2* |
| *Total* | *24* | *Total* | *0* | *Total* | *11* |

*Table 4.1* Numbers and types of responses made by 28 nucleus accumbens neurons to conditioned stimuli presented to rats behaving within the modified go/no-go task. See *Data Analysis* for explanation of the response type classifications. Note that a neuron could exhibit more than one response, for instance, excitation to the reward-predictive conditioned stimulus and inhibition to the aversive-predictive conditioned stimulus.

In contrast to predictions made by the behavioural switching hypothesis, we found that no neuron responded solely to conditioned stimuli that signaled a switch in behaviour. However, approximately one quarter of neurons responding to the conditioned stimulus (8/28) produced 11 responses classified as outcome-switching responses: specifically their activity varied depending on the upcoming outcome and the subsequent switching of the rat's behaviour (see Figure 4.9 for an example response pattern). The number and valences of outcome-switch (4 excitatory, 1 inhibitory) *versus* outcome-no switch (4 excitatory, 2 inhibitory) responses were approximately equal.

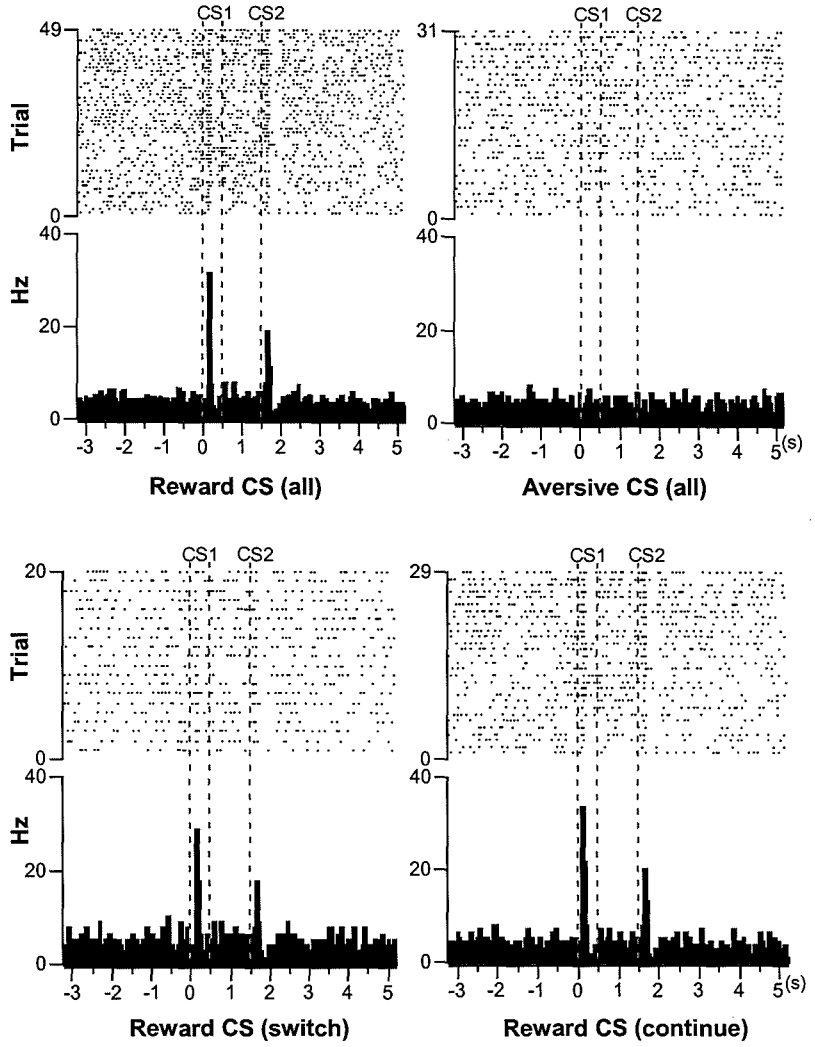



*Figure 4.8* Example from a single neuron classified as exhibiting an excitatory outcome-predicting response. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the conditioned stimulus predicted the rewarding outcome *(light; top left)*, where the conditioned stimulus predicted the aversive outcome *(tone; top right)*, where the conditioned stimulus that predicted the rewarding outcome was followed by a switch in the rat's behaviour *(light; bottom left)*, and where the conditioned stimulus that predicted the rewarding outcome was followed by no switch in the rat's subsequent behaviour *(light; bottom right)*. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 and the onset of conditioned stimulus-2, respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=40ms for all histograms. Repeated-measures ANOVA revealed that this response was significantly influenced by the upcoming outcome ($F_{(2,114)}$=10.839, $p<0.001$), not by the subsequent switching behaviour of the rat ($F_{(2,114)}$=0.038, $p=0.945$) or by a combination of subsequent switching and the upcoming outcome ($F_{(2,114)}$=1.847, $p=0.168$). Pairwise comparisons revealed that this response significantly predicted the rewarding ($p<0.001$) but not aversive ($p=0.591$) outcomes.

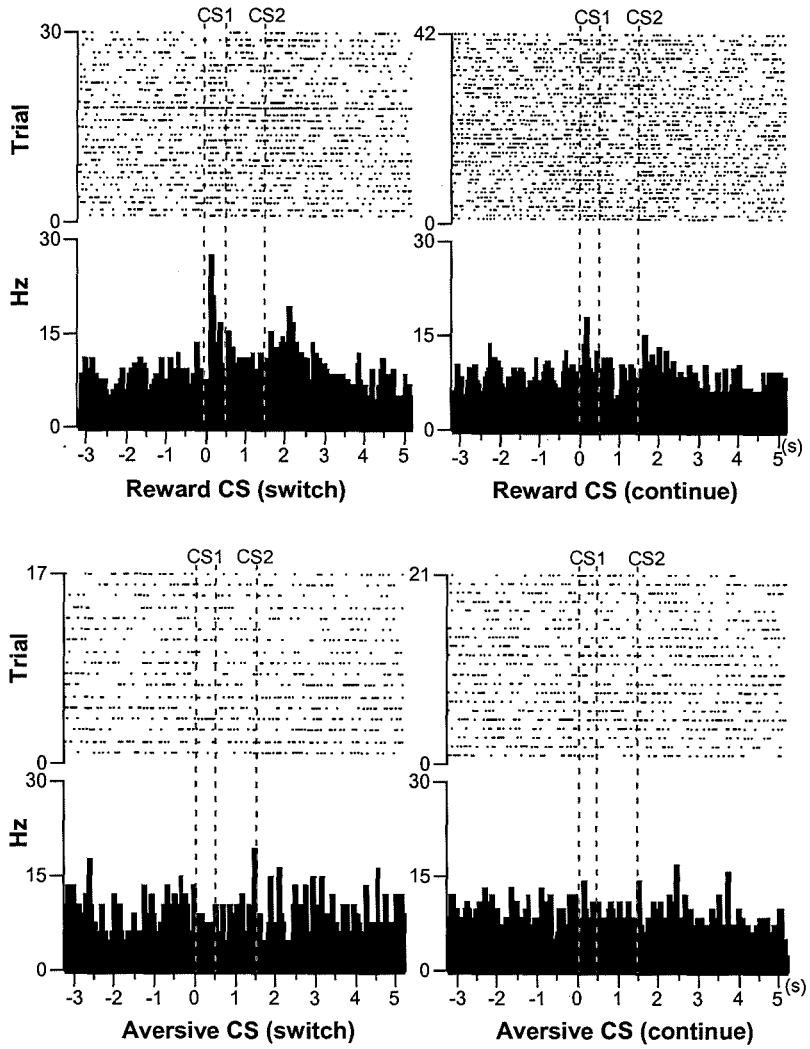



*Figure 4.9* Example from a single neuron classified as exhibiting an excitatory outcome-switching response. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the rat switched behaviour following presentation of the reward-predictive conditioned stimulus *(light; top left)*, made no switch in behaviour following presentation of the reward-predictive conditioned stimulus *(light; top right)*, switched behaviour following presentation of the aversive-predictive conditioned stimulus *(tone; bottom left)*, and made no switch in behaviour following presentation of the aversive-predictive conditioned stimulus *(tone; bottom right)*. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 and the onset of conditioned stimulus-2, respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=40ms for all histograms. Repeated-measures ANOVA revealed that this response was significantly influenced by the upcoming outcome alone ($F_{(2,174)}$=3.069, *p=0.049*), a combination of upcoming outcome and subsequent switching ($F_{(2,174)}$=4.954, *p=0.008*), but not by the rat's subsequent switching alone ($F_{(2,174)}$=1.089, *p=0.339*). Pairwise comparisons revealed that this response significantly predicted the subsequent rewarding outcome and a subsequent switch in behaviour by the rat (*p<0.001)*.

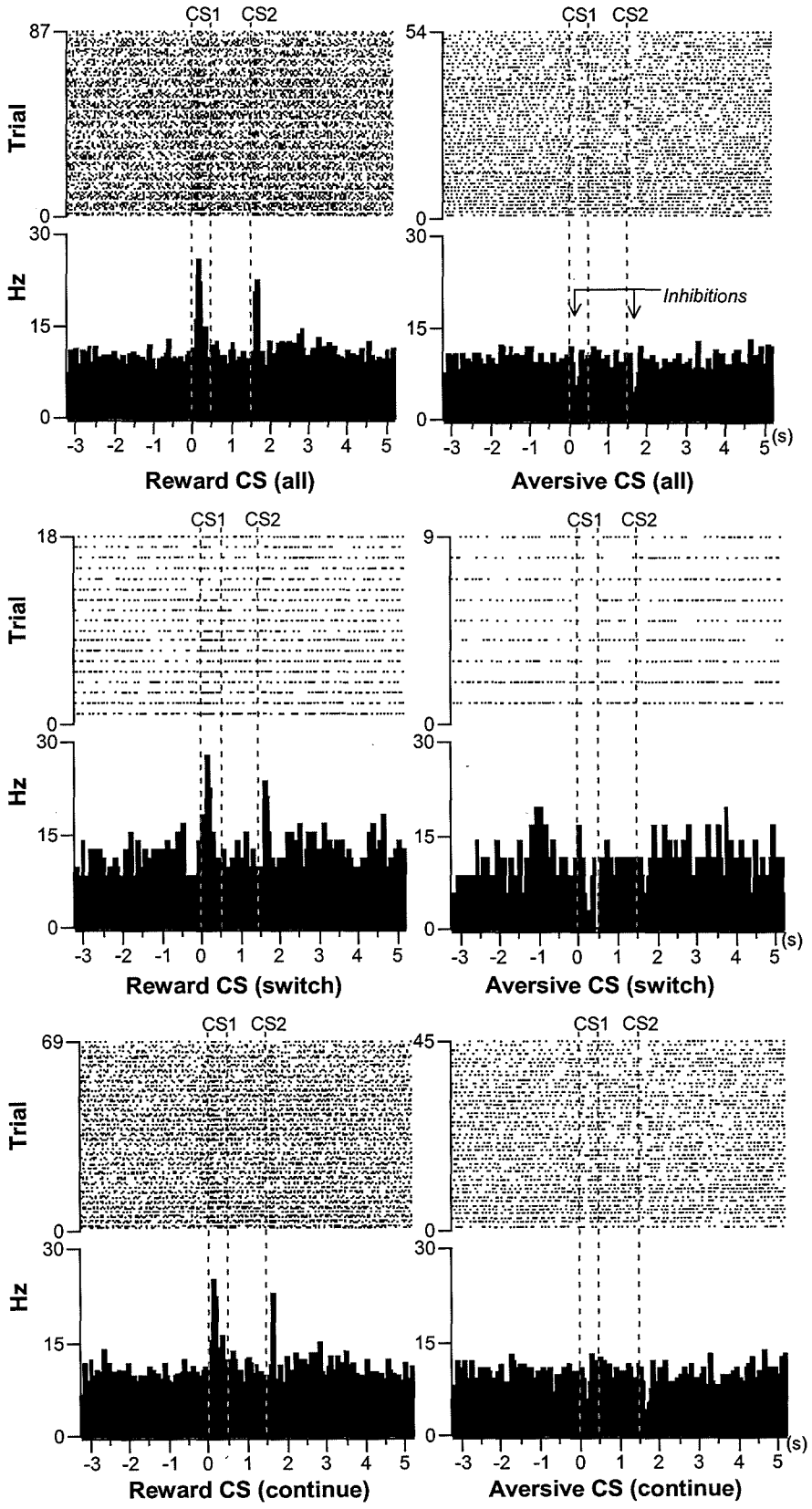



*Figure 4.10* (See legend on next page)

*Figure 4.10 (previous page)* Example from a single neuron classified as exhibiting an inhibitory aversive-predictive response and an excitatory reward-predictive response. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the conditioned stimulus predicted the rewarding outcome *(tone; top left)*, where rats made a switch in behaviour following presentation of the reward-predictive conditioned stimulus *(tone; middle left)*, trials where rats made no switch in behaviour following presentation of the reward-predictive conditioned stimulus *(tone; bottom left)*, all trials where the conditioned stimulus predicted the aversive outcome *(light; top right)*, trials where rats made a switch in behaviour following presentation of the aversive-predictive conditioned stimulus *(light; middle right)* and trials where rats made no switch in behaviour following presentation of the aversive-predictive conditioned stimulus *(light; bottom right)*. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 and the onset of conditioned stimulus-2, respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=40ms for all histograms. Repeated-measures ANOVA revealed that this response was significantly influenced by the upcoming outcome ($F_{(2,226)}$=22.633, $p<0.001$), not by the subsequent switching behaviour of the rat ($F_{(2,226)}$=0.109, $p=0.897$) nor by a combination of subsequent switching and the upcoming outcome ($F_{(2,226)}$=0.551, $p=0.577$). Pairwise comparisons revealed that this response significantly predicted the aversive ($p=0.038$) and rewarding ($p<0.001$) outcomes.

Additionally, we compared the magnitude of outcome-prediction and behavioural switching effects. We found that across all responsive neurons (n=28) the magnitude of the outcome-prediction effect (median $eta^2$=0.051) was approximately 2.5 times that of the effect of behavioural switching (median $eta^2$=0.020), and approximately 3.5 times that of an interaction effect between outcome-prediction and behavioural switching (median $eta^2$=0.014).

*MOST NEURAL RESPONSES WERE TO REWARD-RELATED RATHER THAN AVERSIVE-RELATED STIMULI*

We next wanted to establish if there was a bias in the type of outcome predicted by nucleus accumbens neural responses since the behavioural switching hypothesis suggests that reward-related and aversive-related stimuli would evoke equivalent activity. As shown in Table 4.1 the majority of outcome-predicting

responses (19/24) and outcome-switch responses (8/11) were in anticipation of rewarding rather than aversive outcomes. All reward outcome-predicting responses were excitatory (although one reward-no switch response was inhibitory), and were usually short (<200ms), phasic bursts (see example in Figure 4.8). In contrast, aversive outcome-predicting responses were excitatory (2/5) or inhibitory (3/5; see Figure 4.10 for an example response pattern). Typically, when neurons responded to aversive-predictive conditioned stimuli they also responded to the reward-predictive conditioned stimuli (4/5 neurons). In most of these cases (3/4) the neurons responded with differential valence to the reward-predictive (excitation) *versus* aversive-predictive (inhibition) conditioned stimuli (see Figure 4.10). When we considered the average response of all neurons (n=82; see Figure 4.11) we found that the response was largely modulated by the reward-predictive properties of conditioned stimuli. However, in switch *versus* no-switch conditions the excitatory response to the reward-predictive conditioned stimulus was enhanced and the response to the aversive-predictive conditioned stimulus reversed in sign.

There was also a bias for neurons to respond to the delivery of rewarding *versus* aversive outcomes (18/23 of neurons responding to outcome delivery responded to saccharin rather than to quinine and 2 neurons responded to both outcomes; see Table 4.2). Responses to rewarding (13/20) and aversive (3/5) outcomes tended to be inhibitory (see Figure 4.12 for example of a reward response). Figure 4.13 indicates that the population of neurons responding with significant inhibition during saccharin consumption did so in a manner that co-varied with licking rate. The population of neurons that exhibited significant excitation during saccharin consumption responded maximally prior to the most vigorous bout of licking. Importantly, these firing patterns were not related to individual lick actions. In spite of this subgroup of responses, the overall average neural population response (n=82) revealed no significant response to rewarding ($F_{(1,81)}$=0.762, *p=0.385*) or aversive outcomes ($F_{(1,81)}$=1.779, *p=0.186*).

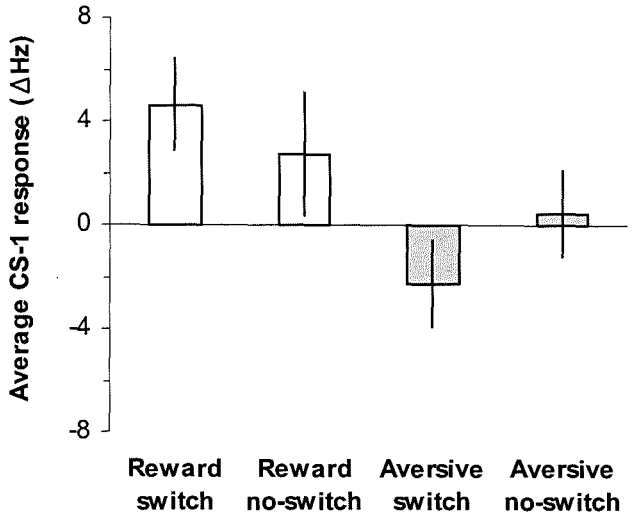



*Figure 4.11* Population responses of neuronal activity to conditioned stimulus-1 (CS-1) minus baseline activity (ΔHz) on trials within which the reward or aversive-predictive conditioned stimulus caused a switch or no-switch in the rat's subsequent behaviour from all neurons (n=82; ±95% CI). Repeated-measures ANOVA revealed that the population conditioned stimulus-1 response was significantly influenced by the upcoming outcome alone ($F_{(2,739)}$=13.452, *p<0.001*, $eta^2$=0.032), a combination of upcoming outcome and subsequent switching ($F_{(2,739)}$=3.387, *p=0.038*, $eta^2$=0.008), but not by the rat's subsequent switching alone ($F_{(2,739)}$=0.210, *p=0.791*). Pairwise comparisons between epochs at each outcome-switching combination revealed a significant inhibitory response when the rats switched their behaviour in anticipation of an aversive outcome (*p=0.028*), significant excitation when rats made no switch in their responding in anticipation of reward (*p=0.*043) and an enhanced excitatory response by a factor of 1.7 when the rats did switch their behaviour in preparation of reward (*p<0.001*).

*REWARD OUTCOME-PREDICTING RESPONSES DID NOT DIRECTLY TRIGGER CONDITIONED LICK RESPONSES*

We have demonstrated that neurons predominantly responded to the outcome-predictive properties of conditioned stimuli rather than their behavioural switching properties. It is possible that reward outcome-predictive responses encoded an association between the conditioned stimulus and the rats' subsequent conditioned response to lick for reward. If this were the case, then these neural responses would signal a specific switch from rats' on-going behaviour (even if it

was operant-licking) to trigger a conditioned lick response. Since almost all conditioned stimuli were followed by a conditioned response, we were unable to compare neural responses to conditioned stimuli that were followed by a conditioned response *versus* no-conditioned response on a neuron-to-neuron basis. However, we were able to compare neural activity over the population of reward outcome-predicting responses between these conditions. Given that all reward outcome-predicting responses were excitatory, if the apparent reward outcome-predicting responses triggered conditioned lick responses, then the population activity would show greater excitation to conditioned stimuli followed by conditioned licking *versus* those that were not. However, we found this population exhibited a significant neural response to the reward-predictive conditioned stimulus that did not differ between conditioned stimuli followed by a conditioned response *versus* those followed by no conditioned response (see Figure 4.14). Indeed, the response was marginally *greater* in the no-conditioned response *versus* conditioned response condition (11.64Hz *versus* 9.96Hz, respectively).

| Rewarding outcome | Aversive outcome | Rewarding and Aversive outcome |
|---|---|---|
| *18* | *3* | *2* |

*Table 4.2* Numbers and types of responses by 23 nucleus accumbens neurons to outcome-delivery in rats behaving within the modified go/no-go task. See *Data Analysis* for explanation of the response type classifications.

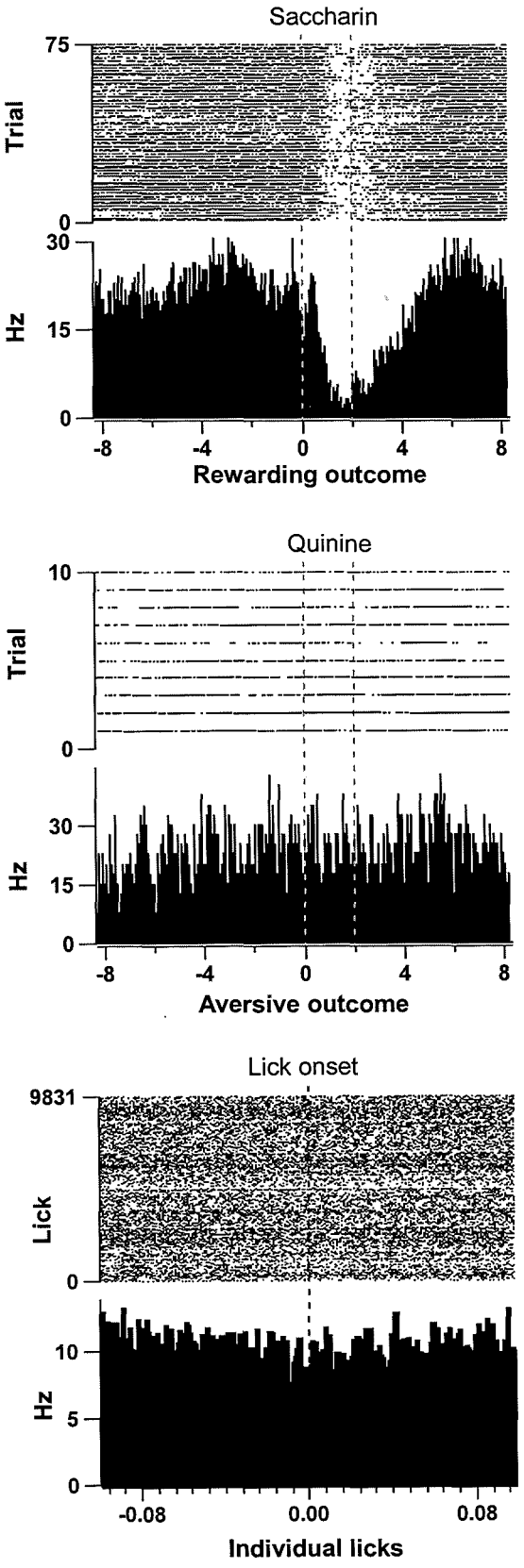



*Figure 4.12* Example from a single neuron exhibiting an inhibitory response during delivery of saccharin solution (rewarding outcome) and no response to delivery of quinine solution (aversive outcome) or to individual lick movements. Rasters and histograms show average firing rate (Hz) of the neuron relative the onset of the rewarding outcome (*top*), aversive outcome (*middle*) and individual licks (*bottom*). *A/B*: Dashed lines at 0 and 2 seconds represent the onset and offset of saccharin (*top*) and quinine (*middle*) delivery, respectively. Bin size=40ms. Rasters from bottom to top show each trial from the session start to end. Repeated-measures ANOVA revealed this response was dependent on the outcome ($F_{(1,83)}$=24.800, *p<0.001*) and pairwise comparisons showed that the response was specific to the rewarding (*p<0.001),* not aversive (*p=0.455)* outcome. *Bottom*: Dashed line at 0 seconds represents the onset of every individual lick within session. Bin size= 1ms. The time included in the histogram (0.1s pre- and post-lick onset) was chosen to avoid contamination of sampling overlapping lick onsets (maximum of the average lick rate during reward consumption~10Hz (see Figure 4.5)). The inhibition following reward was, therefore, not due to artefacts recorded when the rat contacted the reward spigot with its tongue.

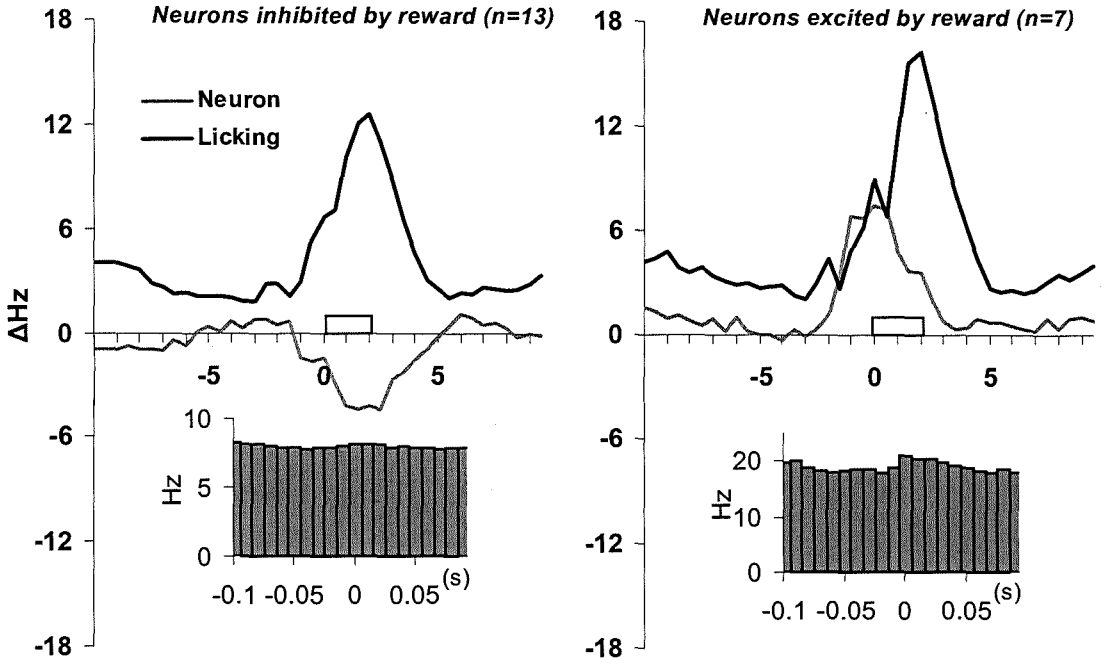



*Figure 4.13* Population responses of neuronal activity (ΔHz; grey line) and lick rates (Hz; black line) from neurons that exhibited significant inhibitory (*left*; n=13) and excitatory (*right*; n=7) responses during consumption of the rewarding saccharin outcome. The white boxes indicate reward delivery. *Insets*: Population histograms of neuronal response relative to individual licks. The time included in the histogram (0.1s pre- and post-lick onset) was chosen to avoid sampling overlapping lick onsets.

Secondly, we evaluated whether reward outcome-predicting neural responses were predictive of the vigour of licking made immediately after presentation of the conditioned stimulus-1. We found no correlation between neural response magnitude to the reward-predictive conditioned stimulus (z-scores) and anticipatory licking rate between the conditioned stimulus-1 presentation and reward delivery (z-scores) across all trials from neurons that exhibited a reward outcome-predicting response (Spearman's rho= 0.023, *p=0.420*, n=1222 trials from 19 reward outcome-predicting neural responses). Therefore, it seems that the majority of nucleus accumbens neural responses (reward outcome-predicting) in our sample encoded upcoming outcome type and did not seem to

facilitate behavioural switching or correlate with the subsequent conditioned licking response.

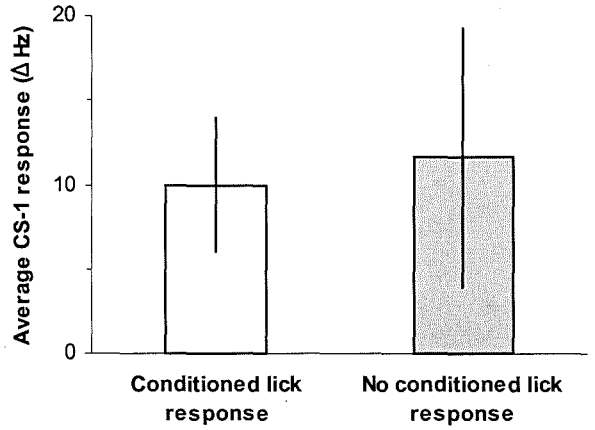



*Figure 4.14* Average neural population responses to the reward-predictive conditioned stimulus-1 (CS-1) minus baseline activity (ΔHz; ±95% CI) when the reward-predictive conditioned stimulus was followed by a conditioned lick response *versus* no-conditioned lick response (n=15 neurons). This neural population consisted of neurons that exhibited a significant response to the reward-predictive conditioned stimulus-1 and were recorded during sessions within which there were trials containing conditioned lick responses and no-conditioned lick responses. N.B. On average there were 60.53 (±9.54, 95% CI) and 2.16 (±0.97, 95% CI) trials per session containing conditioned lick responses and no-conditioned lick responses, respectively. This population exhibited a significant response to the reward-predictive conditioned stimulus ($F_{(2,28)}$=14.350, *p<0.001*) that was not differential between 'conditioned lick response' *versus* 'no-conditioned lick response' conditions ($F_{(2,28)}$=0.652, *p=0.529*). Independent repeated-measures ANOVA's revealed significant excitation to reward-predictive conditioned stimuli followed by a conditioned lick response ($F_{(2,28)}$=14.991, *p<0.001*) and those followed by no-conditioned lick response ($F_{(2,28)}$=7.923, *p=0.003*).

## DISCUSSION

We answered our initial questions by demonstrating that (1) approximately two-thirds of neural responses to conditioned stimuli appeared to encode the upcoming outcome, approximately one-third of responses processed a combination of outcome-prediction and behavioural switching information, and no neuron encoded the presence or absence of a behavioural switch, irrespective of the upcoming outcomes. The population response to conditioned stimulus-1 exhibited a strong outcome-predictive response that was additionally modulated by behavioural switching; (2) over three-quarters of conditioned stimulus responses were to conditioned stimuli signaling the availability of the rewarding, rather than aversive outcome. Every reward outcome-predicting response was excitatory in valence. There was a mixture of excitatory and inhibitory aversive outcome-predicting responses.

### ALL NEURAL RESPONSES TO CONDITIONED STIMULI ENCODED OUTCOME-PREDICTING INFORMATION

Our data are consistent with many previous reports that single neurons in the nucleus accumbens respond to conditioned stimuli predictive of positive or negative outcomes, as well as to the outcomes themselves (Bowman et al. 1996; Carelli et al. 2000; Chang et al. 1998; Cromwell and Schultz 2003; Hassani et al. 2001; Hollerman et al. 1998; Nicola et al. 2004b; Peoples and West 1996; Setlow et al. 2003; Shidara et al. 1998; Tremblay et al. 1998; Wilson and Bowman 2004a). Moreover, our data replicate previous reports of a bias in the valence of responses to reward-predictive conditioned stimuli towards excitations *versus* inhibitions (Carelli and Ijames 2001; Hollerman et al. 1998; Nicola et al. 2004b; Wilson and Bowman 2004a), a bias in the valence of responses to rewarding outcome delivery towards inhibition *versus* excitation (Chang et al. 1998; Nicola et al. 2004c; Peoples and West 1996; Taha and Fields 2005; Wilson and Bowman 2004a) and a bias towards the proportion of neurons responding to

reward-predictive *versus* aversive-predictive conditioned stimuli (Williams et al. 1993).

However, it has recently been reported that nucleus accumbens neurons responded more to aversive-predictive than reward-predictive conditioned stimuli in rats performing a go/no-go task for sucrose/quinine, and in cats within a non-operant environment (Setlow et al. 2003; Yanagimoto and Maeda 2003). This suggests that nucleus accumbens neurons are not inherently biased to process reward-related information. In order to explain the differences in reward versus aversive responses among studies we considered variations in the spatial distribution of sampled recording sites since there is evidence of a rostrocaudal gradient in the shell of the nucleus accumbens for processing appetitive *versus* aversive reactions (Reynolds and Berridge 2003, 2002). However, this is unlikely to explain the low numbers of aversive responses seen in our study, since our electrode tracts often extended into caudal areas of the shell. Moreover, we found responses to aversive-predictive conditioned stimuli at both anterior (+1.7mm from bregma) and posterior (+0.7mm from bregma) sites. In contrast, the recording sites of Setlow *et al.* were less caudal to ours and were confined to the core subregion of the nucleus accumbens.

A more plausible explanation for the presence of a bias towards reward-related responses in our study is that rats were exposed to more reward *versus* aversive trials throughout training and testing. Consequently, the conditioned stimulus was more predictive of the outcome in reward *versus* aversive trials. Indeed, stronger reward *versus* aversive conditioning was reflected in rats' behaviour since there were fewer 'incorrect no-go' responses in reward trials than 'incorrect go' responses in aversive trials (see Figure 4.4).

REWARD OUTCOME-PREDICTING RESPONSES DID NOT CORRELATE WITH CONDITIONED LICK RESPONSES

Although outcome-predicting responses did not correlate with the rat's subsequent switching behaviour they might have triggered conditioned lick responses. We found limited evidence against this hypothesis since the average population response of reward outcome-predicting responses did not differentiate between trials within which reward-predictive conditioned stimuli were followed by a conditioned lick response *versus* trials when they were not. Indeed, independent analyses revealed significant neural responses to conditioned stimulus-1 in both the conditioned lick response and no-conditioned lick response conditions, with a trend of *greater* excitation in the no-conditioned lick response *versus* conditioned response condition. Additionally, we found no correlation between the magnitude of reward outcome-predicting response and the vigour with which the rat licked following conditioned stimulus-1 presentation. However, we recognize that the number of trials in which the reward-predictive conditioned stimulus failed to evoke a conditioned lick response is low. Therefore, although the neural response to the reward-predictive conditioned stimulus did not differentiate between trials in which a conditioned lick response occurred versus when it did not, the statistical power available from this sample is low. Moreover, we cannot exclude the possibility that nucleus accumbens activity triggers motor responses in a probabilistic way depending on the pattern of activity in other neural structures.

Recently, single neurons in the ventral striatum were recorded during learning within a go/no-go task (Setlow et al. 2003). Setlow et al. (2003) divided their neuronal population into neurons that started to make discriminative responses between appetitive and aversive stimuli prior to acquisition of the discrimination *versus* neurons that only showed differential neuronal activity after acquisition. Characteristics of the former type of neuron included a lack of modulated activity in the presence of a subsequent conditioned response and a relatively high baseline-firing rate. It seems that our sample was akin to that of the first type of neuron described by Setlow *et al.* Conversely, other workers, e.g. Schultz, seem to sample more of the second type (motor-related) of neurons (Hassani et al.

2001; Hollerman et al. 1998; Tremblay et al. 1998). Possible explanations for these differences include neuronal sampling biases due to differences in neurophysiology equipment, species differences and/or neuronal sampling during different stages of learning between studies.

Indeed, there are previous reports that nucleus accumbens (Bowman et al. 1996; Carelli and Ijames 2001; Hassani et al. 2001; Hollerman et al. 1998; Setlow et al. 2003; Shidara et al. 1998; Wilson and Bowman 2004a), midbrain dopaminergic (Schultz 1998), ventral pallidal (Tindell et al. 2004) and basolateral amygdala (Schoenbaum et al. 1999) neurons seem to respond to the motivational but not motor aspects of stimuli. In this regard, the typical response pattern seen in our neurons (excitations to conditioned stimulus-1 and 2 with greater magnitude to conditioned stimulus-1) are similar to response patterns of dopamine neurons in the macaque (excitations to two different, consecutively presented conditioned stimuli, with greater magnitude of response to the conditioned stimulus presented earliest within the trial (Schultz et al. 1993)) and ventral pallidal neurons in the rat (Tindell et al. 2004). Here, we have provided additional evidence that many nucleus accumbens neural responses to conditioned stimuli do not correlate with subsequent behavioural switching.

### A PROPORTION OF NEURAL RESPONSES ENCODED OUTCOME-PREDICTIVE AND BEHAVIOURAL SWITCHING INFORMATION

Our task design allowed us to analyse neural responses to conditioned stimuli that triggered a switch in the rat's subsequent behaviour, involving a *reallocation of behavioural resources,* as previously defined by Redgrave et al. (1999). Thus, in one type of 'switch' trial rats stopped bar-pressing and reallocated their behavioural resources to spigot-licking, whilst in the other they stopped spigot-licking and reallocated their behavioural resources to avoid the spigot (typically rats moved back or turned away from the spigot). These trials were in contrast to 'no-switch' trials within which there was no change in the allocation of

behavioural resources since the rats continually licked the spigot from pre- to post-conditioned stimulus presentation.

We found no neuron within our sample responded exclusively during switching of the rat's upcoming behaviour. However, nearly one third of neural responses evoked by the conditioned stimulus were determined by an interaction of the anticipated outcome and behavioural switching, as was the activity of all neurons when considered as a population. These responses are interpreted as signaling a switch or 'no-switch' in the rat's subsequent behaviour for a particular outcome. Akin to reward outcome-predicting responses, it is unlikely that these responses triggered conditioned lick responses since both reward-switch and reward-no switch responses were followed by a conditioned lick response. The pattern in the valence and number of 'outcome-switch' (4 excitations + 1 inhibition = 5 responses) *versus* 'outcome-no switch' (4 excitations + 2 inhibitions = 6 responses) demonstrated that approximately equal numbers of neurons encoded outcome-switching *versus* outcome-no switching information. It should be noted that the median magnitude of effect of the outcome-switching interaction, across all responsive neurons, was 3.5 times less than that of the outcome-prediction effect.

These data corroborate with modulations in cue-directed behavioural switching following cell-body lesions of the nucleus accumbens (Bowman and Brown 1998; Reading and Dunnett 1991; Reading et al. 1991), neurochemical lesions of dopaminergic inputs to the nucleus accumbens (Evenden and Carli 1985; Robbins and Koob 1980), and psychopharmacological modulation of dopamine neurotransmission within the nucleus accumbens (Bakshi and Kelley 1991a, b; Cools 1980; Evenden and Robbins 1983a, b; Oades 1985; Robbins and Sahakian 1983; van den Bos and Cools 2003; Yun et al. 2004a). Additionally, our data demonstrate that neural responses in the nucleus accumbens can occur in both the presence *and* absence of a subsequent behavioural switch. Furthermore, it is possible that nucleus accumbens neurons *only* encode

switching information when they also encode outcome-predicting information. Indeed, it seems from our data that behavioural switching has a more limited role than outcome-prediction in nucleus accumbens processing since no neuron solely encoded behavioural switching information, there were fewer outcome-switching than outcome-prediction neurons and outcome-prediction had the greatest magnitude of effect on neural responses to conditioned stimuli.

## CONCLUSIONS

In summary, over two-thirds of conditioned stimulus-responsive nucleus accumbens neurons in our sample responded to the outcome-predictive properties of conditioned stimuli and did not seem to facilitate behavioural switching. However, approximately one third of neural responses were outcome-predictive with additional modulation by the presence or absence of a subsequent behavioural switch. These data suggest a primary functional role for the nucleus accumbens in encoding outcome-predicting information (Cardinal and Everitt 2004; O'Doherty et al. 2004; Parkinson et al. 2000a; Robbins and Everitt 2002; Schultz et al. 2003; Setlow et al. 2002; Setlow et al. 2003) and a more limited role in behavioural switching (Evenden and Carli 1985; Reading and Dunnett 1991; Reading et al. 1991; Robbins and Koob 1980; Robbins and Sahakian 1983; van den Bos and Cools 2003).

## CHAPTER 5

# NEURONS IN DOPAMINE-RICH AREAS OF THE RAT MIDBRAIN PREDOMINANTLY ENCODE THE OUTCOME-RELATED RATHER THAN BEHAVIOURAL-SWITCHING PROPERTIES OF CONDITIONED STIMULI

The work presented in this chapter is currently under review for publication in the *Journal of Neurophysiology*.

**ABSTRACT**

It has been hypothesized that midbrain dopamine neurons respond to sensory stimuli to minimize reward-prediction errors or to facilitate behavioural switching. To test the latter hypothesis we recorded from single neurons in the ventral tegmental area and retrorubral field of rats responding during a modified go/no-go task. Within each trial a conditioned stimulus was predictive of a rewarding (saccharin) or aversive (quinine) outcome and caused a switch or 'no-switch' in the rat's behaviour (see Wilson and Bowman 2005). One-quarter of neurons (33/131) recorded within dopamine-rich areas of the midbrain responded to the conditioned stimulus. Almost half of these (45%) processed the outcome-predictive properties of the conditioned stimulus; the activity of a minority (15%) correlated with an aspect of behavioural switching (mostly responding in the *absence* of a behavioural switch) and one-third (33%) encoded various outcome-switch combinations. The most common response and the average population response was excitation to the reward-predictive conditioned stimulus. Additionally, a proportion of neurons responded during outcome delivery, typically exhibiting inhibition during saccharin consumption. We made no attempt to dichotomize our sample into dopaminergic *versus* GABAergic neurons, since the conventional criteria (waveform length, apomorphine response, firing rate etc.) can result in misclassification of neurons in the midbrain of the alert rat. However, the reward-predictive responses we observed were akin to dopamine responses previously reported in the macaque and to rat nucleus accumbens responses observed within the same task. Conversely, inhibitory reward responses were similar to those previously reported for GABA neurons. We conclude that the pattern of results from our sample is more consistent with the reward-prediction hypothesis than the behavioural-switching hypothesis.

## INTRODUCTION

It has been reported that dopamine neurons respond with a phasic short-latency excitatory burst to stimuli predictive of 'better-than-expected' reward and with inhibition to stimuli predictive of 'worse-than-expected' reward or of aversive stimuli (Fiorillo et al. 2003; Mirenowicz and Schultz 1996; Schultz 1998; Tobler et al. 2003; Tobler et al. 2005). The bias in numbers of responses towards reward-predictive *versus* aversive-predictive stimuli indicate that dopamine neurons might signal the value of motivational stimuli in relation to reward (Mirenowicz and Schultz 1996; Schultz 1998). However, it has also been established that dopamine neurons respond to novel or non-rewarding salient stimuli that trigger orienting reactions, with both behavioural and neural responses habituating over repeated presentations (Horvitz et al. 1997; Schultz 1998; Steinfels et al. 1983; Strecker and Jacobs 1985). Thus, it has been hypothesized that dopamine neurons might not encode reward expectancy, but instead signal the presence of stimuli that are motivationally salient (e.g. novel, aversive or rewarding) (Horvitz 2000; Redgrave et al. 1999b). Additionally, Redgrave et al. (1999b) have proposed that dopamine responses might facilitate a switch in the animal's attention and/or behaviour to salient stimuli. Indeed, manipulations to the mesoaccumbens or mesostriatal dopamine systems can modulate switching of rats' behavioural strategies in order to gain reinforcers or to avoid aversive stimuli (Bakshi and Kelley 1991a, b; Evenden 2002; Evenden and Carli 1985; Evenden and Robbins 1983b; Robbins and Koob 1980; Robbins and Watson 1981; van den Bos and Cools 2003).

We sought to test the hypothesis that dopamine neurons uniformly respond to motivationally salient stimuli that trigger a switch in behaviour. To do so, we recorded the activity of single neurons in the ventral tegmental area and in the retrorubral field of rats responding within a modified go/no-go task. In this task, presentation of conditioned stimuli predicted a rewarding or aversive outcome and caused a switch or no-switch in the rat's subsequent behaviour (this task has

been used previously to test the role of nucleus accumbens neurons in behavioural switching (Wilson and Bowman 2005)). We hoped as well to determine whether responses reflected motivational valence *versus* motivational salience by comparing the sign of responses to reward-predictive *versus* aversive-predictive conditioned stimuli. Since current electrophysiological and pharmacological techniques do not allow for accurate identification of dopamine neurons in the ventral tegmental area of the rat (Kiyatkin and Rebec 1998), we initially assessed the prevalence of outcome-predictive *versus* behavioural-switching response types across all neurons in dopamine-rich areas and subsequently assessed the similarity between these responses with those from identified dopamine neurons of macaque monkeys (Schultz 1998).

## METHODS

### SUBJECTS

Twenty-one Listar Hooded adult male rats (Harlan UK), housed in quadruplets on a light 12h: dark 12h light cycle, weighed 399g (± 35g, 95% CI) when training began. During experimental training and testing rats were free to consume water from 4-5PM each weekday and from Friday 4PM until Sunday afternoon. This did not cause the rats' body weights to fall below 85% of their free-drinking weight. After electrode implantation rats were housed in isolation. All procedures conformed the United Kingdom 1986 Animals (Scientific Procedures) Act using guidelines outlined in the "Handbook of Laboratory Animal Management and Welfare" (Wolfensohn and Lloyd 1998).

### APPARATUS

#### *BEHAVIOUR*

Rats were trained in sound-attenuated testing chambers (34cm x 29cm x 25cm; Med Associates Inc., St Albans, VT) each fitted with a video camera (Santec Smart Vision, model VCA 5156, Sanyo Video Vertrieb GmbH Co., Ahrensburg, Germany). Along one wall of each chamber was a retractable lever (left side), drinking spigot (centre), houselight (top centre) and piezoelectric buzzer (behind spigot; model EW-223A, Med Associates Inc.). Sodium saccharin solution (0.25% w/v) or quinine hydrochloride (0.2% w/v) solution could be pumped out of the drinking spigot at 0.05ml/s from one of two 50ml glass syringes (Rocket, London) by one of two computer controlled syringe pumps (model PHM - 100, Med Associates Inc.). These solutions did not mix since they were pumped through separate lines of tubing. The stiff nature of the steel plungers in the syringes ensured reliable flow rates and precise timing of liquid delivery.

#### *NEUROPHYSIOLOGY*

We used electrode arrays with a movable bundle of four 50μm stainless steel microwires coated in Teflon (tip impedance 0.5-1.5MΩ). We could advance the wires between recording sessions by turning an 80-thread/inch set screw (Small Parts Inc., Miami Lakes, FL, USA) advancing the wires ~317.5 μm/turn. Differential activity from two pairs of wires was amplified, filtered and subsequently processed with the CED 1401™ data acquisition system (Cambridge Electronic Design, Cambridge, UK). The sampling resolution was limited to 2ms of the MED-PC™ system. Detailed descriptions of the neurophysiological apparatus, surgical, histological and spike sorting techniques have been described previously (Wilson and Bowman 2004).

## PROCEDURES

Rats were trained over a period of approximately two months to reach the final stage of training outlined below. Initial training stages were similar to those described previously (Wilson and Bowman, 2005).

### *FINAL TRAINING STAGE*

Rats were trained to make operant responses on a variable ratio-3 schedule to earn saccharin or quinine outcomes. The operant response required by the rat was either bar-pressing or spigot-licking. Ten consecutive trials with the same operant response type constituted a block of trials. The block type (pressing or licking) was sequentially alternated and signaled to the rat by protrusion and withdrawal of the bar, respectively (the first block of trials was randomly assigned as pressing or licking). On each trial, once rats had made between 1-5 operant responses (variable ratio-3 schedule, a random selection of 1-5 responses), a reward-predictive or aversive-predictive conditioned stimulus was presented for 0.5s. The reward-predictive conditioned stimulus was randomly chosen to be presented in ~66% of trials and the aversive-predictive conditioned stimulus in ~33%. The rats were divided into two groups of conditioned stimulus modality with one group receiving a tone reward-predictive conditioned stimulus using the

piezoelectric buzzer, and the other group receiving a light reward-predictive conditioned stimulus using the houselight. From the rats that we successfully recorded neurons within dopamine areas of the midbrain there were equal numbers (n=6) that received a tone *versus* the houselight as the reward-predictive conditioned stimulus.

Subsequent to presentation of the conditioned stimulus (*conditioned stimulus-1*) there was a 1s-delay, followed by a second presentation of the same conditioned stimulus (*conditioned stimulus-2*). This double presentation allowed rats to prepare their 'go/no-go' response. If a lick ('go' response) was made within 2s of the conditioned stimulus-2 onset the rat received 0.1ml rewarding saccharin solution (when the conditioned stimulus was reward-predictive) or 0.1ml aversive quinine solution (when the conditioned stimulus was aversive-predictive). Outcome delivery lasted two seconds along with the continued presentation of conditioned stimulus-2. When the rat ended the lick bout after the outcome had been delivered (defined as an inter-lick interval >= 300ms) there was an inter-trial interval lasting 4s that was unsignalled to the rat. If the rat did not lick within 2s of conditioned stimulus-2 onset an 'error' was recorded, the conditioned stimulus turned off, an unsignalled inter-trial interval of 2.5s was initiated and rats were advanced to a new trial. Figure 5.1 illustrates this final testing stage of the modified go/no-go task.

*SURGERY*

An electrode array was permanently implanted onto the skull of each rat with the electrode targeted stereotaxically at the ventral tegmental area (-5.30mm posterior and 0.8-1mm lateral to bregma; 7.4mm-7.7mm ventral to skull surface).

*NEUROPHYSIOLOGICAL RECORDING*

Rats were given 5-7 days to recover from surgery. Neural recording during the modified go/no-go task lasted approximately four weeks per rat.

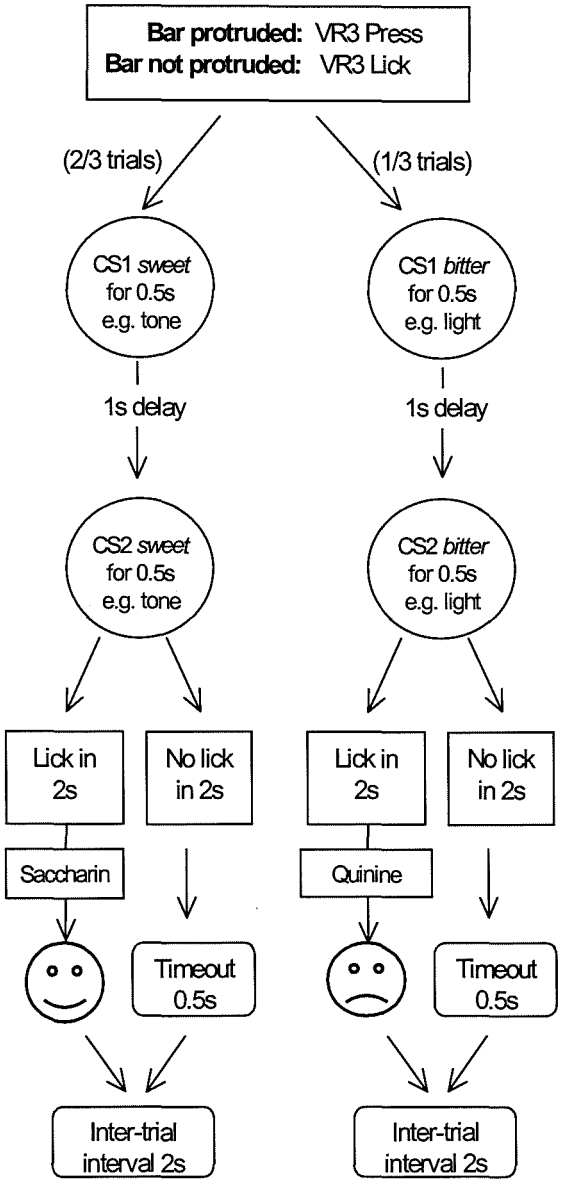



*Figure 5.1* Schematic representation of behavioural procedures of a given trial during the modified go/no-go task. *From top:* Rats worked through alternating blocks of trials (ten trials/block). Within each trial, rats were required to make operant responses (either pressing or licking depending on the block) on a variable ratio-3 schedule. Following responding, the reward-predictive conditioned stimulus-1 (CS sweet; randomly selected for 2/3 trials) or aversive-predictive conditioned stimulus-1 (CS bitter; randomly selected for 1/3 trials) was presented for 0.5s. Following a 1s delay a second presentation of the stimulus (conditioned stimulus-2) was made. If the rat licked the spigot (a 'go' response) within 2s of this stimulus rewarding saccharin solution or aversive quinine solution was delivered, respectively. The trial stopped following the end of the lick bout (defined as an inter-lick interval > 300ms) after the outcome had been delivered, and an inter-trial interval of 2s commenced (unsignaled to the rat). If the rat did not lick the spigot (a 'no-go' response) within 2s of the conditioned stimulus-2 presentation there was an unsignalled timeout of 2.5s. The timeouts ensured 'go' and 'no-go' trials were approximately equivalent in length.

*HISTOLOGY*

Following neurophysiological recording rats were killed by overdose with 0.7ml Dolethal™ (200 mg/l pentobarbitone sodium BP; Univet Ltd., Oxford, UK) and perfused intracardially with 0.1% phosphate buffer saline followed by a fixative (4% paraformaldehyde in 0.1M phosphate buffer). 50-µm thick sections were cut

on a freezing microtome, collected in 0.1M phosphate buffer, and 1:4 sections stained for Nissl bodies and tyrosine hydroxylase. These sections were then analysed under a light microscope and damage from electrode tracts were mapped onto standardised sections of the brain (Paxinos and Watson 1997).

## DATA ANALYSIS

### *BEHAVIOUR*

We restricted our behavioural analysis to the testing sessions in which activity from neurons estimated to be in the tyrosine hydroxylase-stained areas of the midbrain had been sampled (n=63 sessions). We independently performed repeated measures ANOVA on the average percentage of trials within which rats made a 'go' response, and the average rate rats licked during outcome delivery with two within-subject factors, *Operant response type* (licking *versus* pressing) and *Outcome type* (rewarding saccharin *versus* aversive quinine).

### *NEUROPHYSIOLOGY*

*Spike sorting.* We re-sorted spikes offline by performing cluster analysis on the waveforms in the data set (Spike2$^{TM}$). When identical neurons were recorded over consecutive testing days (as identified by visual inspection of the waveform shape/duration, interspike interval histogram, average firing rate, and event-related activity) we used only the data from the session within which the rat completed the most trials.

*Windows for spike counts.* On the basis of previous recordings of dopamine neurons (Schultz 1998) we predicted there would be responses to conditioned stimulus-1 within 200ms of its onset. Therefore we calculated the average firing rate (Hz) of each neuron 0-200ms pre- ('baseline' window) and 0-200ms post- (response window) conditioned stimulus-1 onset. We also compared the average firing rate –100 to 100ms relative to trial onset with firing during the 2s of

outcome delivery (we used this baseline window in an attempt to maintain similarity with Wilson and Bowman (2005)).

*Assignment of trial types.* As has been outlined previously (Wilson and Bowman 2005) we were able to examine the effects of outcome-prediction on neural responses since ~66% of conditioned stimuli were reward-predictive and ~33% aversive-predictive. We were also able to determine if neural activity at the conditioned stimulus-1 correlated with the rat's subsequent switching behaviour, since there were two types of trial where the rats made a subsequent behavioural switch and one type where rats made no switch in behaviour (see Figure 5.2).

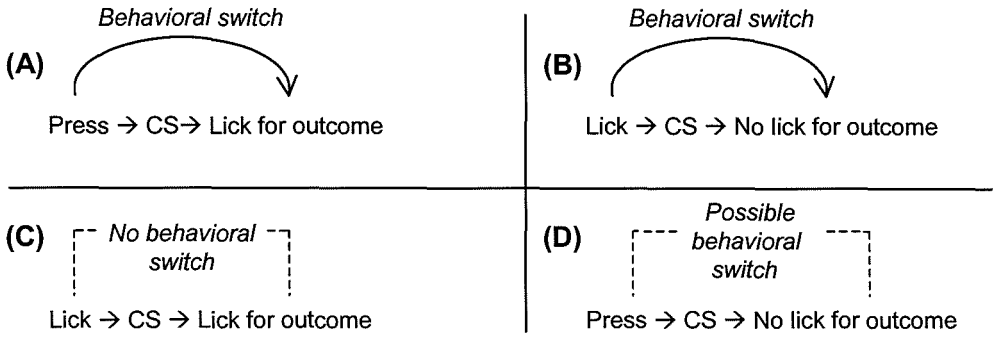



*Figure 5.2* Schematic representation of the trial types classified within our operational definition of behavioural switching. *(A)* Rats switched behaviour from bar-pressing to spigot-licking following presentation of most reward-predictive and some aversive-predictive conditioned stimuli (CS), respectively. *(B)* Rat switched behaviour from spigot-licking approach behaviour to avoidance of the spigot following presentation of few reward-predictive and most aversive-predictive conditioned stimuli, respectively. *(C)* Rats maintained pre-conditioned stimulus licking (and thus made no switch in behaviour) to gain outcome delivery subsequent to presentation of most reward-predictive, and over half of aversive-predictive, conditioned stimuli. *(D)* Rats did not switch behaviour to lick the spigot for outcome delivery. However, we were unable to define the onset and offset of bar-pressing behaviour (often the rat's paw remained on the bar without fully depressing it) and so could not determine whether rats switched to a different behaviour or made no switch in behaviour and continued to bar-press. Consequently, these trials were dropped from the analysis.

*Classification of response type.* We performed mixed design repeated measures ANOVAs with pairwise comparisons on spike frequency (Hz) across each trial per neuron over the baseline versus response time windows around the presentation of conditioned stimulus-1 (repeated-measures factor, *Epoch*) comparing conditioned stimuli that predicted aversive *versus* rewarding outcomes (between-group factor, *Outcome type*) and conditioned stimuli that caused the rat to make a switch *versus* no-switch response (between-group factor, *Switching type*). Neurons were classified as exhibiting an outcome-predicting response (reward outcome-predicting or aversive outcome-predicting) to the conditioned stimulus when there was a significant *Epoch*Outcome type* interaction (*p<=0.05)* and a significant pairwise comparison (*p<=0.05)* between the baseline and response epoch time windows at the reward-predictive or aversive-predictive conditioned stimulus-1. Similarly, neurons were classified as exhibiting a switching response (switch or no-switch) or an outcome-switching response (reward-switch, reward-no switch, aversive-switch, aversive-no switch) to the conditioned stimulus when there was a significant ANOVA effect (*Epoch*Switching type* or *Epoch*Outcome type*Switching type*, respectively, *p<=0.05*) and a significant pairwise comparison (*p<=0.05)* between baseline and response windows at each type of response (switch, no-switch and reward-switch, reward-no switch, aversive-switch, aversive-no switch, respectively). It was possible for one neuron to have more than one type of response.

Mixed design repeated measures ANOVAs with pairwise comparisons were also performed on spike frequency (Hz) across each trial per neuron over a baseline (-1s to +1s trial onset) and outcome response time windows (repeated-measures factor, *Epoch*) comparing aversive *versus* rewarding outcomes (between-group factor *Outcome type*). Neurons were classified as exhibiting an outcome response when there was a significant *Epoch*Outcome type* interaction effect (*p<=0.05*) and a significant pairwise comparison between baseline and response firing rates to aversive or rewarding outcome types (*p<=0.05)*.

In cases where repeated measures ANOVA was performed the Hunyh-Feldt correction was used to decrease the effect of heterogeneity of variance and the Sidak test was used to adjust for multiple pairwise comparisons. Details of additional analyses are presented in the appropriate figure legends and were performed using Microsoft Excel 2000™ and SPSS 10.0 for Windows™. Rasters and histograms were presented using Spike 2™.

*Correlation of neural response with individual licking movements.* Since consummatory licking movements were rhythmic and stereotyped, we could not identify an appropriate time window to compare lick-related responses with activity within a baseline time window. Therefore, we divided up the neural activity into 8 bins from lick onset to offset to the subsequent lick onset. Repeated-measures ANOVA was performed on the average firing rate across bins over the recording session for each neuron. For each neuron we then calculated the $r^2$ value summarizing the proportion of shared variability between the temporal pattern of licking (main effect of *Bin*) and neural activity. We used this as an index of the degree to which each neuron encoded orofacial movements. We used the estimated partial $\eta^2$ values from the *Epoch*Outcome type* calculation in the ANOVA that was used to detect outcome-related responses. We used this as an index of the magnitude of outcome-related activity. In order to determine whether the apparent outcome-related activity was due to motor signals we correlated the lick-related $r^2$ values with the outcome-related $\eta^2$ values using the non-parametric, Spearman rank correlation test.

## RESULTS

### BEHAVIOUR

Similar to our preceding experiment (Wilson and Bowman 2005) rats licked significantly faster during saccharin *versus* quinine delivery (see Figure 5.3). The low licking rates during quinine delivery suggest that the rats found quinine aversive. Rats also exhibited differential numbers of 'go' responses to earn saccharin *versus* aversive outcomes, indicating they had learnt the predictive value of conditioned stimuli (see Figure 5.4). We classified three different switching trial types dependent on whether the rat switched its behaviour subsequent to conditioned stimuli presentation: licking to no-licking (switch), pressing to licking (switch) or continued licking (no-switch). These patterns were evident in population licking and pressing histograms (see Figure 5.5).

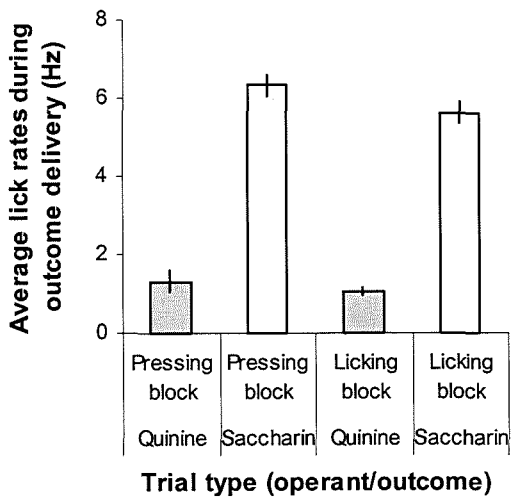



*Figure 5.3* Average licking rates (Hz; ±95% CI) during quinine and saccharin delivery following pressing and licking operant responses for rats (n=55/63 sessions from 12 rats during successful recording from 131 neurons within tyrosine hydroxylase-stained areas of the midbrain (8 sessions were excluded in which there were no trials containing quinine delivery within the session)). Repeated-measures ANOVA revealed rats licked significantly faster to delivery of saccharin *versus* quinine ($F_{(1,54)}$=1381.757, $p<0.001$).

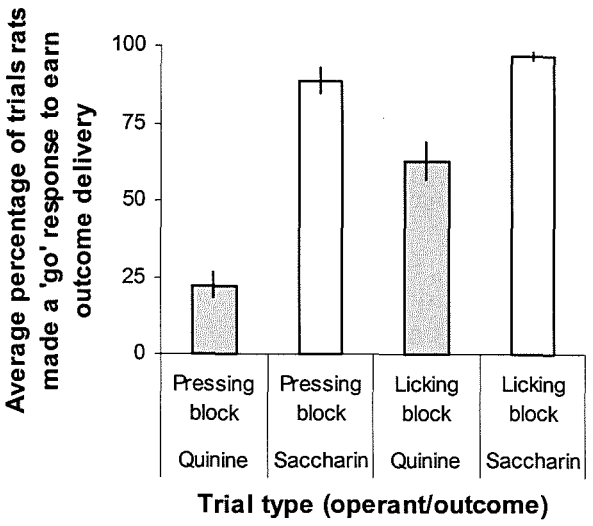



*Figure 5.4* Average percentage of trials (±95% CI) in which rats (n=63 sessions from 12 rats) made 'go' responses to earn quinine and saccharin delivery following pressing and licking operant responses during successful recording from 131 neurons within tyrosine hydroxylase-stained areas of the midbrain. Rats made fewer 'go' responses for quinine delivery under both responding conditions ($F_{(1,62)}$=539.812, $p<0.001$).

NEUROPHYSIOLOGY

*MANY NEURONS RESPONDED SOLELY TO THE OUTCOME-PREDICTIVE PROPERTIES OF CONDITIONED STIMULI*

We successfully recorded from 228 neurons in 15 rats responding within the modified go/no-go task. By reconstructing the location of each electrode tract, and estimating the dorsal-ventral distance of the recording wires at each recording session, we estimated that 131 neurons from 12 rats were within tyrosine hydroxylase-stained areas of the midbrain (11/12 electrodes were within the ventral tegmental area; see Figure 5.6). One-quarter of neurons within these dopamine-rich areas (33/131; 25%) were classified by our criteria as having 41 different responses to conditioned stimulus-1. Almost half of these responsive neurons (15/33; 45%) exhibited differential activity to the reward-predictive *versus* aversive-predictive conditioned stimulus-1 that did not correlate with the rats' subsequent behavioural switching. Indeed, the most common response to conditioned stimulus-1 was excitation to the reward-predictive conditioned stimulus (see Table 5.1; see Figure 5.7 for an example response). Conversely, responses to the aversive-predictive conditioned stimulus were exclusively

inhibitory. Responses to the reward-predictive conditioned stimulus-1 were similar in sign, latency and duration to those of nucleus accumbens neurons within the same task (Wilson and Bowman 2005).

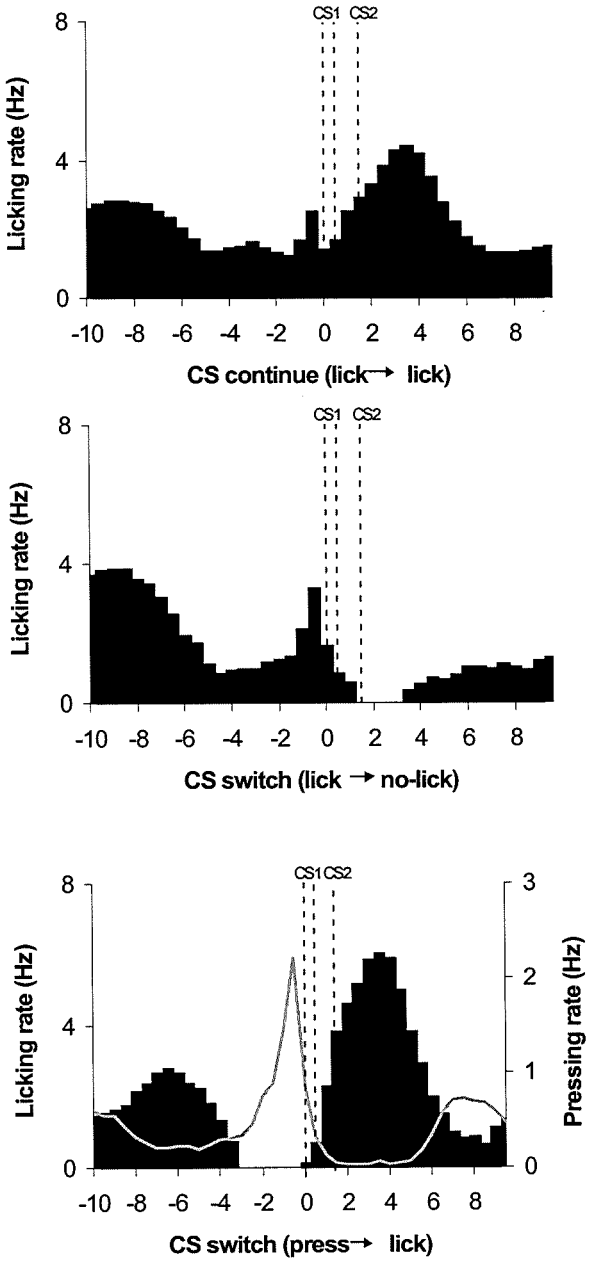



*Figure 5.5* Average licking rates (Hz) by rats (n=63 sessions from 12 rats) relative to conditioned stimulus-1 (CS1) and conditioned stimulus-2 (CS2) presentation during trials that were classified as (*top*) 'no-switch' since operant licking was continued following presentation of conditioned stimulus-1, (*middle*) 'switch' trials within which operant licking was aborted following presentation of conditioned stimulus-1, and (*bottom*) 'switch' trials within which rats switched from operant bar-pressing to spigot-licking following presentation of conditioned stimulus-1. Bin size=500ms.

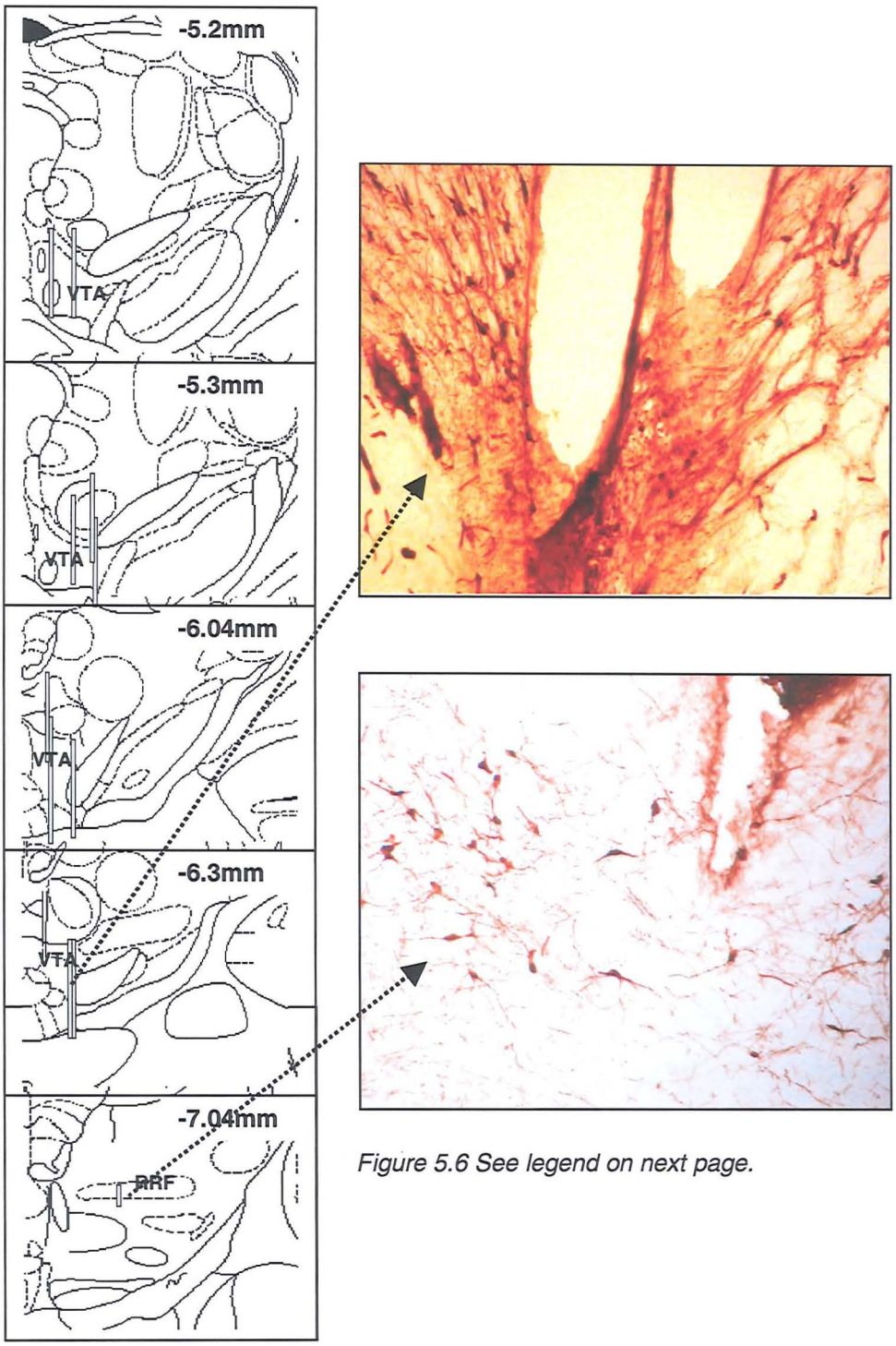



*Figure 5.6 See legend on next page.*

*Figure 5.6 (previous page) Left:* Approximate placements of recording wires within each rat where successful recording took place (n=12). Each overlapping diagram represents a coronal section referenced to bregma (Paxinos and Watson 1997) and each grey rectangle depicts the approximate location of microwires per rat. Rats had microwires extending into tyrosine hydroxylase-stained areas within the ventral tegmental area (11/12 rats) and retrorubral area (n=1 rat; -7.04mm relative to bregma). *Right:* Photographs taken under light microscopy of tyrosine hydroxylase-stained brain sections from three rats within which damage from microwires can be seen beside tyrosine hydroxylase-stained neurons. The location of microwires relative to the tyrosine hydroxylase-stained neurons and the predicted dorsal-ventral distance wires had advanced at each recording session allowed us to estimate whether each neuron was above, below or within the tyrosine hydroxylase-stained neuronal area. Although there were wires within tyrosine hydroxylase-stained areas over a range of anterior-posterior and medio-lateral sites, there were too few statistically significant responses and recording locations to have the requisite statistical power to compare responses between anatomical locations throughout the ventral tegmental area. Illustration adapted from Paxinos & Watson (1997). Abbreviations: VTA (ventral tegmental area), RRF (retrorubral field/A8).

| CS-1 response | Excitation | Inhibition |
|---|---|---|
| *Reward* | 12 | 1 |
| *Aversive* | 0 | 4 |
| *Switch* | 1 | 0 |
| *No-switch* | 4 | 2 |
| *Reward-switch* | 3 | 4 |
| *Reward-noswitch* | 2 | 2 |
| *Aversive-switch* | 2 | 1 |
| *Aversive-noswitch* | 2 | 1 |

*Table 5.1* Responses made by 33 neurons within dopamine-rich areas of the midbrain to the conditioned stimulus-1 (CS-1) during the modified go/no-go task. See *Data Analysis* for explanation of the response type classifications. Note that a neuron could exhibit more than one response, for instance, excitation to the reward-predictive conditioned stimulus and inhibition to the aversive-predictive conditioned stimulus.

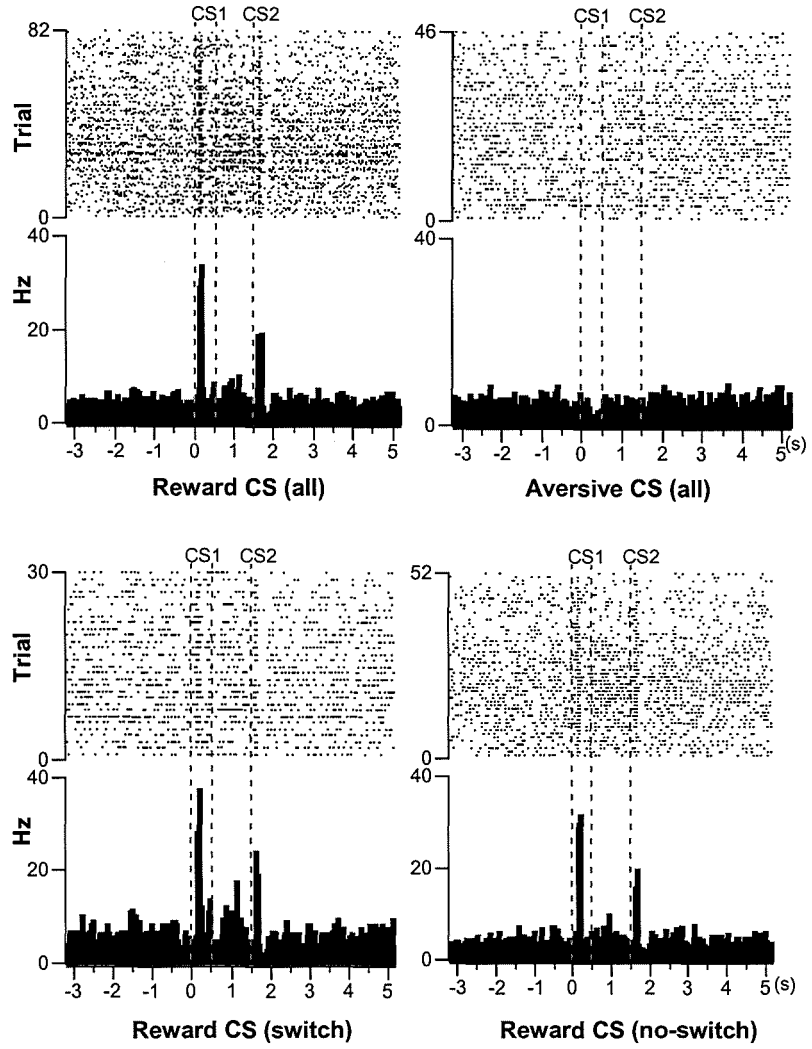



*Figure 5.7* Example from a single neuron classified as exhibiting an excitatory reward-predictive response to the conditioned stimulus-1. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the conditioned stimulus predicted the rewarding outcome *(light; top left)*, where the conditioned stimulus predicted the aversive outcome *(tone; top right)*, where the conditioned stimulus that predicted the rewarding outcome was followed by a switch in the rat's behaviour *(light; bottom left)*, and where the conditioned stimulus that predicted the rewarding outcome was followed by no switch in the rat's subsequent behaviour *(light; bottom right)*. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 (CS1) and the onset of conditioned stimulus-2 (CS2), respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=50ms for all histograms. Repeated-measures ANOVA revealed that this response was significantly influenced by the upcoming outcome ($F_{(1,104)}$=72.348, *p<0.001*, $\eta^2$=0.410) but not by the subsequent switching behaviour of the rat ($F_{(1,104)}$=0.952, *p=0.331*, $\eta^2$=0.009) nor by a combination of subsequent switching and the upcoming outcome ($F_{(1,104)}$=0.005, *p=0.946*, $\eta^2$=0.000). Pairwise comparisons revealed that this response significantly predicted the rewarding (*p<0.001*) but not aversive (*p=0.354*) outcome.

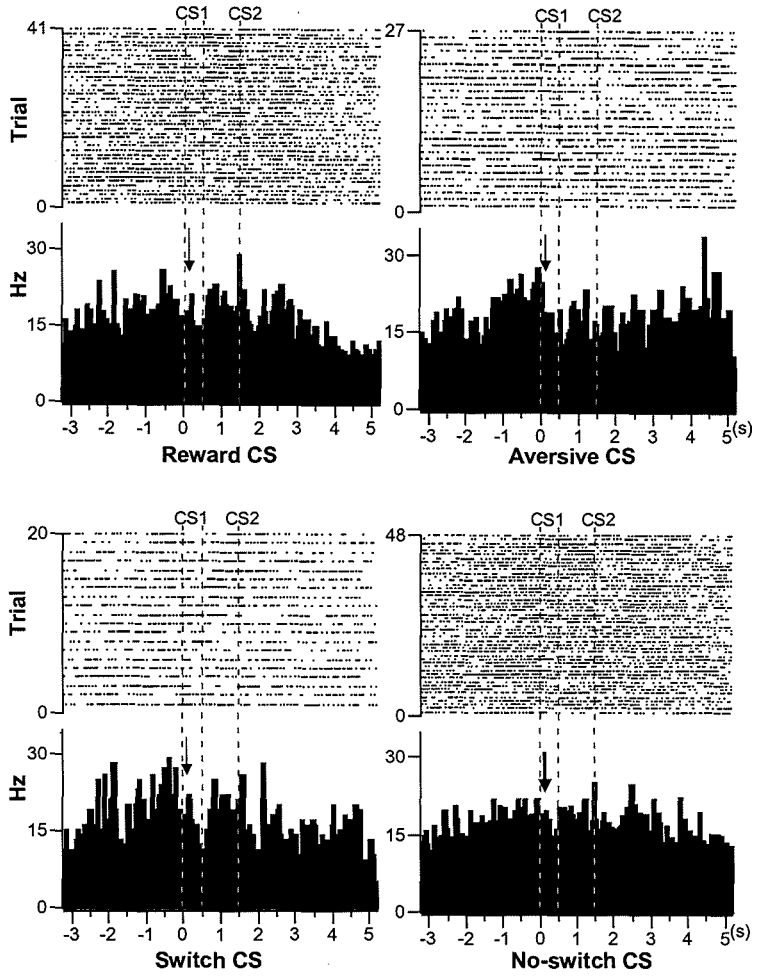



*Figure 5.8* Example from a single neuron classified as exhibiting an inhibitory 'no-switch' response to the conditioned stimulus-1. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the conditioned stimulus predicted the rewarding outcome *(light; top left)*, where the conditioned stimulus predicted the aversive outcome *(tone; top right)*, where rats switched behaviour following presentation of the conditioned stimulus *(tone and light; bottom left)*, and where rats made no switch in behaviour following presentation of the conditioned stimulus *(tone and light; bottom right)*. Arrows depict approximate bins within which response activity was calculated (0-200ms post-conditioned stimulus-1 onset) and the arrow in bold shows the statistically significant response. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 (CS1) and the onset of conditioned stimulus-2 (CS2), respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=50ms for all histograms. Repeated-measures ANOVA revealed that this response was weakly influenced by the upcoming outcome alone ($F_{(1,53)}$=5.046, *p=0.029*, $\eta^2$=0.087), the rat's subsequent switching behaviour alone ($F_{(1,53)}$=7.752, *p=0.007*, $\eta^2$=0.128) but not by a combination of upcoming outcome and subsequent switching ($F_{(1,53)}$=3.524, *p=0.066*, $\eta^2$=0.062). Pairwise comparisons revealed that this response did not significantly predict the subsequent rewarding (*p=0.135*) or aversive outcome (*p=0.095*). However, the response was present when the rat subsequently made 'no-switch' in behaviour (*p=0.017*) but not when they did make a switch (*p=0.141*).

*ACTIVITY OF A MINORITY OF NEURONS CORRELATED WITH BEHAVIOURAL SWITCHING*

A small proportion of neurons (5/33; 15%) exhibited responses to conditioned stimulus-1 that differed between switching *versus* 'no-switching' of the rats' subsequent behaviour. However, in contrast to the predictions made by the switching hypothesis, there was a bias towards neural responses in 'no-switch' (n=6) *versus* 'switch' conditions (n=1) (see Table 1). Moreover, switching-related responses were subtle (see Figure 5.8 of the statistically strongest switching-related response). Finally, one-third of neurons responded to a combination of outcome and switching information (11/33; 33%; see Figure 5.9 for an example response), and in some cases, independently to the outcome-predictive and behavioural switching properties of conditioned stimulus-1 (2/33; 1%). However, there was no bias towards a particular type of 'outcome-switching' response (see Table 1).

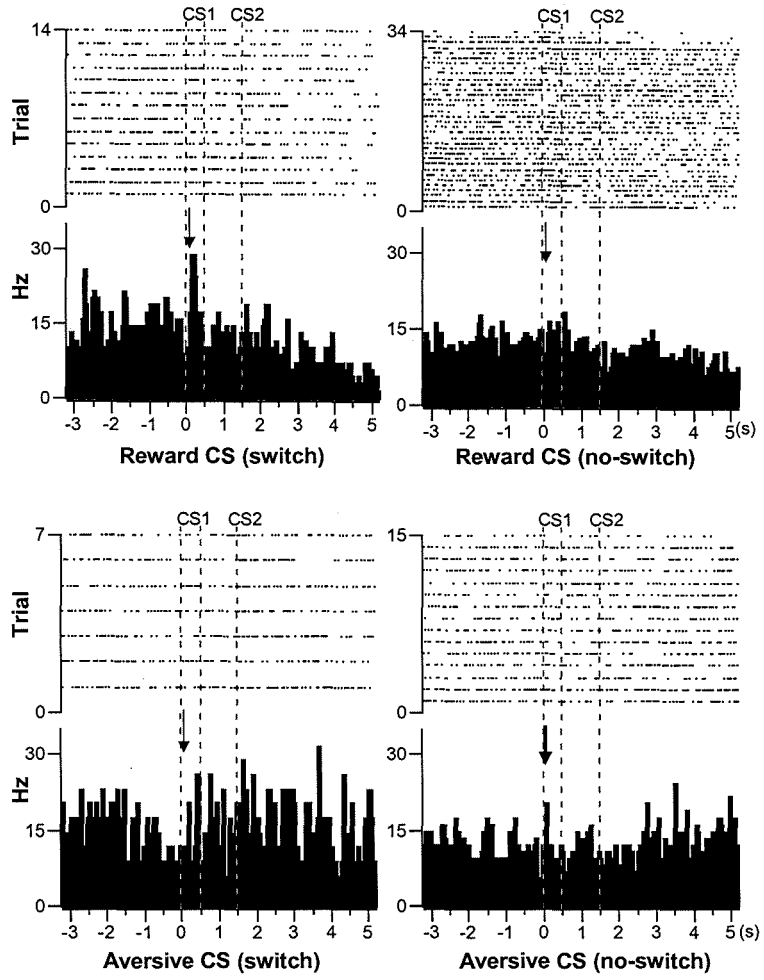



*Figure 5.9* Example from a single neuron classified as exhibiting an excitatory aversive-no switch response to the conditioned stimulus-1. Rasters and histograms show average firing rate of the neuron (Hz) relative to conditioned stimulus-1 on all trials where the conditioned stimulus predicted the rewarding outcome and was followed by a behavioural switch *(tone; top left)*, where the conditioned stimulus predicted the rewarding outcome and was followed by no behavioural switch *(tone; top right)*, where the conditioned stimulus predicted the aversive outcome and was followed by a behavioural switch *(light; bottom left)* and where the conditioned stimulus predicted the aversive outcome and was followed by no behavioural switch *(light; bottom right)*. Arrows depict approximate bins within which response activity was calculated (0-200ms post-conditioned stimulus-1 onset) and the arrow in bold shows the statistically significant response. Dashed lines at 0, 0.5, 1.5 seconds represent the onset and offset of conditioned stimulus-1 (CS1) and the onset of conditioned stimulus-2 (CS2), respectively. Rasters from bottom to top show each trial from the session start to end. Bin size=50ms for all histograms. Repeated-measures ANOVA revealed that this response was not significantly influenced by the upcoming outcome ($F_{(1,81)}$=0.779, *p=0.380*, $\eta^2$=0.010) but was by the subsequent switching behaviour of the rat ($F_{(1,81)}$=5.259, *p=0.024*, $\eta^2$=0.061) and by a combination of subsequent switching and the upcoming outcome ($F_{(1,81)}$=4.587, *p=0.035*, $\eta^2$=0.054). Pairwise comparisons revealed that this response was modulated by the aversive-predictive conditioned stimuli that were followed by no behavioural switch (*p=0.049*).

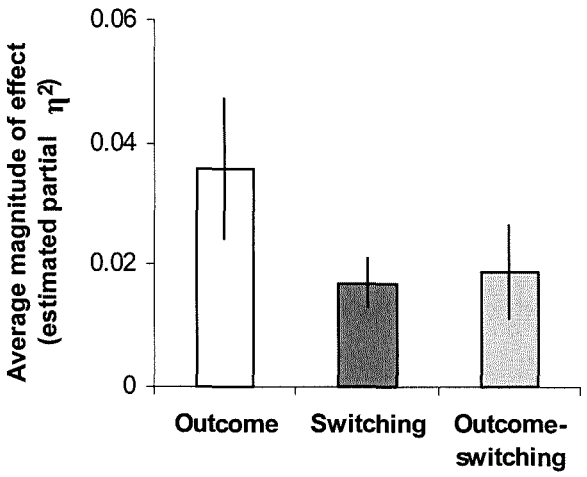



*Figure 5.10* Average magnitude of effect ($\eta^2$) across all neurons (n=131) for *Outcome*Epoch*, *Switching*Epoch* and *Outcome*Switching*Epoch* ANOVA effects. The non-parametric Wilcoxon signed rank test revealed that there was a significantly greater magnitude of effect by the outcome-predictive properties of conditioned stimuli *versus* their switching (*p=0.014*) or outcome-switching (*p=0.001*) properties.

*AVERAGE ACTIVITY HAD STRONGER ENCODING OF OUTCOME-PREDICTION THAN BEHAVIOURAL SWITCHING*

When we analysed the sample of neurons recorded from tyrosine hydroxylase-stained areas (n=131) we found that upcoming outcome had a greater magnitude of effect on neural activity after the conditioned stimulus-1 than behavioural switching or the combination of upcoming outcome and behavioural switching (see Figure 5.10). Moreover, there was an average response by these neurons to the reward-predictive conditioned stimulus-1 that was not modulated by rats' subsequent behavioural switching (see Figure 5.11). The group of neurons that responded to the outcome-predictive properties conditioned stimuli (n=17) exhibited phasic excitations to both conditioned stimuli and phasic inhibitions to aversive-predicting conditioned stimuli (see Figure 5.12). Indeed, the population of neurons with significant responses to the reward-predictive conditioned stimulus-1 (n=13) produced phasic excitations to both conditioned stimulus-1 and 2 that were strikingly similar to the phasic excitations exhibited by the population of nucleus accumbens neurons that responded to reward-predictive conditioned stimuli (n=19), reported previously (Wilson and Bowman 2005) (see Figure 5.13) and to dopamine responses to conditioned stimuli in the macaque (Schultz 1998; Schultz et al. 1993). We visually estimated that this ventral tegmental area population response had an onset latency of ~70ms and duration of ~140ms,

which is comparable to conditioned stimulus responses previously recorded from dopamine neurons in the macaque (onset latency<100ms, burst duration<200ms (Schultz 1998)).

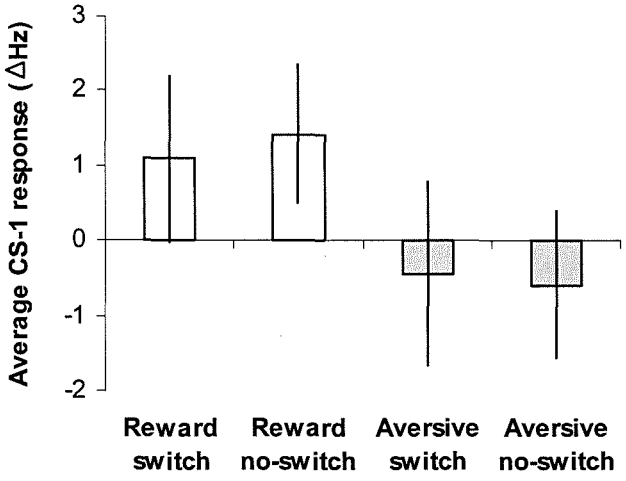



*Figure 5.11* Average population response of neuronal activity to conditioned stimulus-1 (CS-1) minus baseline activity (ΔHz) on trials within which the conditioned stimulus-1 predicted the rewarding or aversive outcome and caused a switch or 'no-switch' in the rat's behaviour (n=524 trials (4 trial types for 131 neurons); ±95% CI). Repeated-measures ANOVA revealed that the population conditioned stimulus-1 response was significantly influenced by the upcoming outcome alone ($F_{(1,520)}$=10.523, *p=0.001*, $\eta^2$=0.020) but not by the rat's subsequent behavioural switching alone ($F_{(1,520)}$=0.031, *p=0.861*, $\eta^2$=0.000) or by a combination of upcoming outcome and subsequent switching ($F_{(1,520)}$=0.190, *p=0.663*, $\eta^2$=0.000). Pairwise comparisons revealed that this response significantly predicted the rewarding (*p=0.001*) but not aversive (*p=0.186*) outcomes.

*NEURAL RESPONSES DURING OUTCOME DELIVERY*

We found that over one-third of neurons responded differentially to saccharin *versus* quinine outcome delivery (50/131; 38%). The majority of responses (37/53; 70%) were inhibitions during saccharin consumption (see Table 2 and Figure 5.14 for an example response). In cases where neurons responded to rewarding *and* aversive outcomes (3/131), they did so with differential valence. Our sample of neurons exhibited an inhibitory response during saccharin and quinine delivery (see Figure 5.15). Although the response appeared stronger to

saccharin *versus* quinine this fell short of statistical significance (*p=0.093*). There was no detectable correlation between the magnitudes of lick-related changes in neural activity and the magnitudes of outcome-related responses (see *Data Analysis*; Spearman's rho=0.090, *p=0.533*, n=50).

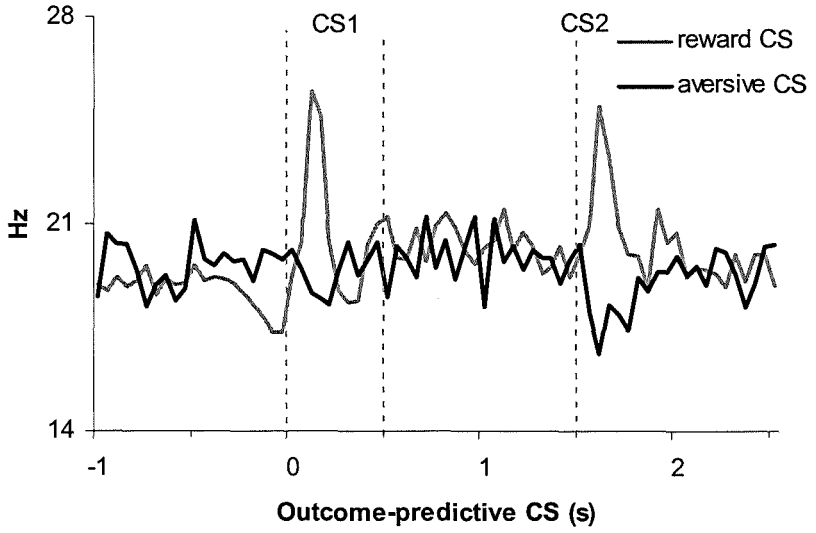



*Figure 5.12* Population response of all neurons responding to the outcome-predictive properties of the conditioned stimulus-1 (n=17) relative to the reward-predictive (grey line) and aversive-predictive (black line) conditioned stimulus-1 (CS1) and 2 (CS2). There appears to be phasic excitatory bursts of activity at both reward-predictive conditioned stimuli and phasic inhibitions at aversive-predictive conditioned stimuli. Bin size=50ms. Repeated-measures ANOVA revealed that the population conditioned stimulus-1 response was significantly influenced by the upcoming outcome alone ($F_{(1,64)}$=24.178, *p<0.001*, $\eta^2$=0.274) but not by the rat's subsequent behavioural switching alone ($F_{(1,64)}$=0.065, *p=0.800*, $\eta^2$=0.001) or by a combination of upcoming outcome and subsequent switching ($F_{(1,64)}$=0.355, *p=0.553*, $\eta^2$=0.006). Pairwise comparisons revealed that there was an average excitatory response to reward-predictive conditioned stimuli (*p<0.001*) and inhibitory response to aversive-predictive conditioned stimuli (*p=0.013*).

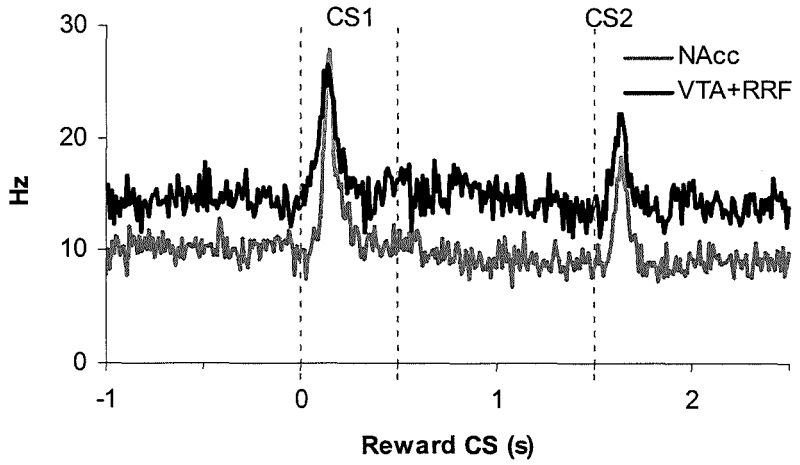



*Figure 5.13* Average population responses of all neurons in the dopamine-rich areas of the midbrain (black line; n=13 (11 neurons in the ventral tegmental area (VTA) and 2 neurons in the retrorubral field (RRF)) and nucleus accumbens (grey line; n=19) that had a significant response to the reward-predictive conditioned stimulus-1. These population responses had an estimated latency of ~70ms and duration of ~140ms in the midbrain and an estimated latency of ~90ms and duration of ~170ms in the nucleus accumbens.

| | **Excitation** | **Inhibition** |
|---|---|---|
| *Saccharin* | 8 | 37 |
| *Quinine* | 4 | 4 |

*Table 5.2* Type and valence of responses to outcome delivery by 50 neurons recorded within the tyrosine hydroxylase-stained areas of the midbrain. A $\chi^2$ goodness-of-fit test revealed that the probability of the four different responses occurring was not equal ($\chi^2$=57.567, $p<0.001$; $H_o$: $p(row_1,column_1)= p(row_1,column_2)= p(row_2,column_1)= p(row_2,column_2)$). An additional $\chi^2$ goodness-of-fit test revealed there was no dependence on frequencies between the rows and columns ($\chi^2$=2.599, $p=0.107$; $H_o$: $p$(cell)= $p$(row)*$p$(column)).

*Figure 5.14* (*Top*) Example from a single neuron classified as exhibiting an inhibitory response during saccharin delivery. Raster and histogram shows average firing rate of the neuron (Hz) relative to saccharin delivery (dashed lines at 0s and 2s represents the onset and offset of saccharin delivery). Raster from bottom to top shows each trial from the session start to end. Bin size=500ms. Repeated-measures ANOVA revealed that this response was differential between saccharin *versus* quinine delivery ($F_{(1,51)}$=8.778, *p=0.005*, $\eta^2$=0.147). Pairwise comparisons revealed that the neuron was inhibited to saccharin delivery (*p<0.001*) but was not responsive to quinine delivery (*p=0.656*). (*Bottom*) Phase histogram of the firing rate of the neuron (spikes/s between closest spikes either side of bin) relative to lick onset (left arrow; bin 1), lick offset (middle arrow; bin 3) and subsequent lick onset (right arrow; bin 8). Bins were divided up across this licking pattern based on the relative mean duration between events (lick onset to lick offset (53ms) *versus* lick offset to next lick onset (81ms)) over the recording session.

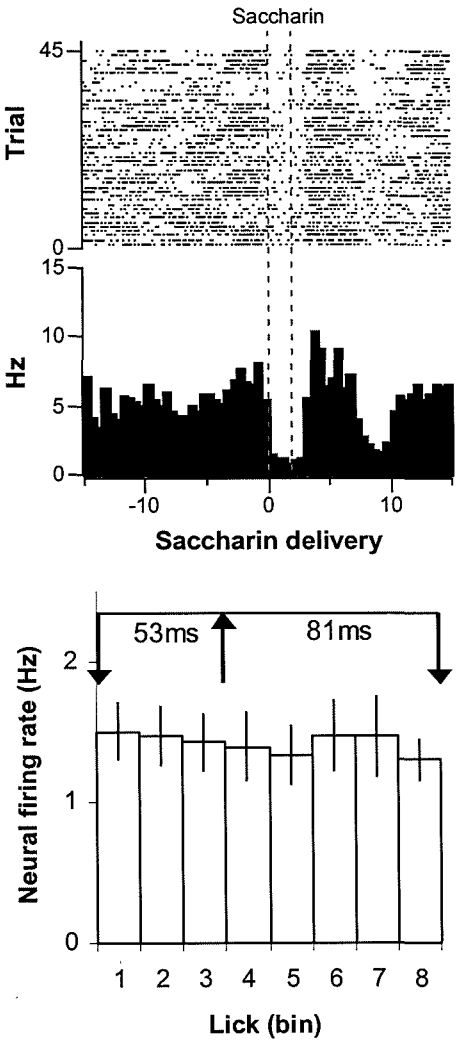
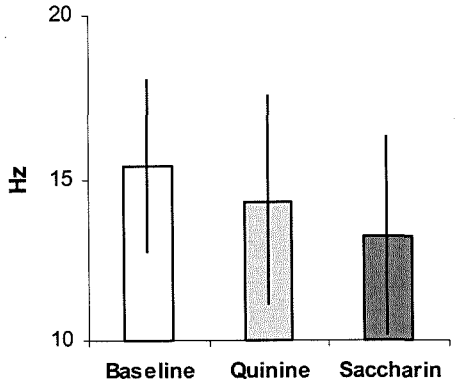



*Figure 5.15* Average population response of the firing rate during 'baseline', quinine delivery and saccharin delivery across all neurons (n=131; ±95% CI). Paired-sample t-tests (two-tailed) revealed that the population response was significantly inhibited during quinine delivery (*p<0.001*) and saccharin delivery (*p=0.001*) relative to baseline. Inhibition was stronger during saccharin *versus* quinine delivery, although this did not reach statistical significance (*p=0.093*).



## DISCUSSION

### SUMMARY

We found that one-quarter of neurons within dopamine-rich areas of the midbrain responded to conditioned stimulus-1 within our modified go/no-go task. Most responses were to the outcome-predictive properties of the conditioned stimulus, predominantly excitations when reward-predictive (12/13) and inhibitions when aversive-predictive (4/4). The population of neurons produced an excitatory response to the reward-predictive conditioned stimulus-1 that appeared consistent with the uniform short-latency phasic activation of classified dopamine neurons previously reported (Schultz 1998). We have demonstrated that these responses only weakly encoded behavioural switching. Additionally, we found that over one-third of neurons responded to outcome delivery with the most common response being inhibition during saccharin consumption.

### RATIONALE FOR NOT CLASSIFYING NEURONS AS DOPAMINERGIC OR GABAERGIC

Some researchers have classified neurons in the ventral tegmental area of alert animals as dopamine-releasing (Hyland et al. 2002; Pan and Hyland 2005; Schultz 1998) on the basis of electrophysiological characteristics (an action potential duration>2ms and firing rate <10Hz with bursts of activity) and pharmacological effects (e.g. inhibitory effects following administration of apomorphine, a dopamine agonist) (Aebischer and Schultz 1984; Freeman et al. 1985; Hyland et al. 2002). Other researchers have classified the remaining non-dopamine neurons as releasing γ-aminobutyric acid (GABA) (Steffensen et al. 1998). However, in the ventral tegmental area of the alert rat, electrophysiological characteristics of recorded neurons do not fall into two distinct clusters representing dopaminergic *versus* non-dopaminergic neurons (Kiyatkin and Rebec 1998). Instead, Kiyatkin and Rebec argue that the electrophysiological characteristics of recorded neurons lie on a continuum, which might reflect the

various subgroups and combinations of peptides, gamma-aminobutyric acid and dopamine present within different neurons of the ventral tegmental area. Indeed, many researchers recording extracellularly from neurons in the ventral tegmental area of the rat have reported no differences in event-related responses between neurons classified as dopaminergic *versus* non-dopaminergic (Kiyatkin and Rebec 2001; Kosobud et al. 1994; Nishino et al. 1987).

There are further difficulties in classifying the pharmacological nature of neurons recorded in the ventral tegmental area of any alert animal. For instance, in anaesthetized rats it has been demonstrated that cortically-projecting dopamine neurons can have 'high' baseline firing rates of >10Hz (Chiodo et al. 1984). Moreover, some *non*-dopamine neurons fulfill the classic criteria for characterization as dopamine neurons, with firing rates <10Hz and action potential durations >2ms (Ungless et al. 2004). It also seems problematic to use these standard classification procedures since 'baseline' firing rates might vary depending on psychological state. For instance, it has been reported that chronic ethanol exposure increased 'baseline' firing rates of classified GABA neurons (Gallegos et al. 1999). Similarly, as recently argued, assessment of waveform duration can differ between anaesthetized *versus* awake recordings due to high-pass filtering used in extracellular recordings from alert animals (Pan and Hyland 2005).

For these reasons we recorded and presented data from all neurons encountered within tyrosine hydroxylase-stained areas of the midbrain. Moreover, we did not test the pharmacological effects of apomorphine on neural activity since it has been demonstrated that many cortically-projecting dopamine neurons are unaffected by administration of apomorphine or iontophoretic application of dopamine (Chiodo et al. 1984); some *non*-dopamine neurons have autoreceptors and are inhibited by apomorphine (see Kiyatkin and Rebec 1998); apomorphine can induce conditioned taste aversion to saccharin (Wise et al. 1976); and testing

the effects of apomorphine might permanently change the characteristics of neurons to be recorded in subsequent sessions.

### RESPONSES TO OUTCOME-PREDICTIVE BUT NOT BEHAVIOURAL-SWITCHING PROPERTIES OF CONDITIONED STIMULI WERE AKIN TO PREVIOUSLY REPORTED DOPAMINE RESPONSES

It has been reported that electrophysiologically classified dopamine neurons respond homogeneously to the reward-predictive properties of conditioned stimuli (Schultz 1998). However, it has been postulated that these responses could initiate a transition in the sequence or chains of behaviour (Redgrave et al. 1999b). Here, the most common response, and the average response, was to the conditioned stimulus predictive of upcoming reward. Although it is possible that some of these responses were from non-dopaminergic neurons, the population response to the reward-predictive conditioned stimulus-1 exhibited a short-latency (~70ms) phasic burst (duration~140ms) that was similar to the population responses of macaque dopamine neurons to conditioned stimuli (onset latency 50-110ms, duration<200ms) (Schultz 1998). Additionally, there was a phasic burst of activity at conditioned stimulus-2, akin to previous reports of dopamine bursts of activity to an instruction cue and trigger stimulus within the same trial of a delayed response task (Schultz et al. 1993).

Importantly, we have demonstrated that these responses do not correlate strongly with behavioural switching, as has been hypothesized (Redgrave et al. 1999b). Although some responses encoded aspects of behavioural switching they did not appear visually similar to previously reported dopamine responses in the macaque. Moreover, response valences were not biased towards switching *versus* 'not-switching' in the manner previously postulated, i.e. there was no bias towards phasic excitations to switch and phasic inhibitions to 'not-switch' (Redgrave et al. 1999b). Since we observed many different types of switching responses, it seems possible that they reflected movement changes between switching conditions, rather than acting to switch the sequence of behaviour *per*

*se* (Bakshi and Kelley 1991a, b; Evenden 2002; Evenden and Carli 1985; Evenden and Robbins 1983b; Redgrave et al. 1999a, b; Robbins and Koob 1980; Robbins and Watson 1981; Takikawa et al. 2004; van den Bos and Cools 2003). Indeed, movement-related activity has previously been found during recordings of classified GABA neurons (Lee et al. 2001).

Responses to the outcome-predictive conditioned stimuli were also consistent with dopamine neurons processing motivational valence (excitations to the reward-predictive and inhibitions to the aversive-predictive conditioned stimulus) (Mirenowicz and Schultz 1996; Schultz 1998) rather than general motivational salience/arousal (Horvitz 2000; Horvitz et al. 1997; Redgrave et al. 1999b; Salamone 1994; Salamone and Correa 2002). However, this finding should be treated with caution since there were too few responses to the aversive-predictive conditioned stimulus (n=4) to generalize to the population.

It seems that some neurons in dopamine-rich areas of the midbrain and the nucleus accumbens (Wilson and Bowman 2005) respond almost identically with phasic bursts of spikes to reward-predictive conditioned stimuli. Unfortunately, we were unable to interpret whether these sets of neurons responded coincidentally or whether neurons in one area excited the neurons of the other area, since the rats between studies had slightly different degrees of training. Indeed, it has recently been found that learning can affect the latency of dopamine responses (Takikawa et al. 2004). Therefore, understanding the interactions between these sets of neurons during outcome-prediction remains a goal for future research.

### MANY RESPONSES WERE INHIBITED DURING REWARD CONSUMPTION

From neurons recorded within the dopamine-rich areas of the midbrain (predominantly within the ventral tegmental area) we found that four-fifths of responses to saccharin delivery were inhibitory. These results are consistent with previous findings that inhibition was the most common response across all

ventral tegmental area neurons during food or sucrose consumption and heroin delivery (Kiyatkin and Rebec 2001; Kosobud et al. 1994; Nishino et al. 1987). It is unlikely that inhibitions in our experiment reflected the sensory properties of reward since the average population response to the consumption of quinine was also inhibitory. Therefore, it is likely that they correlated with consummatory behaviour (although not individual licking movements) or possibly acted to terminate preceding excitatory activity during goal-seeking.

It has previously been demonstrated that classified dopamine neurons are excited to the detection of unpredicted juice delivery (Ljungberg et al. 1991; Mirenowicz and Schultz 1994; Schultz 1998) whereas classified GABA neurons in the rat are predominantly inhibited throughout delivery of ethanol and rewarding brain stimulation (Gallegos et al. 1999; Steffensen et al. 2001). Therefore, it is possible that inhibitory responses to reward found here and reported previously from unclassified neurons (Kiyatkin and Rebec 2001; Kosobud et al. 1994; Nishino et al. 1987) were from GABA neurons. Indeed, only 1/10 of neurons excited solely to the reward-predictive properties of the conditioned stimulus-1 were inhibited during saccharin consumption.

However, we cannot eliminate the possibility that at least some inhibitory reward responses were from dopamine neurons for two reasons: First, it is possible that a proportion of the GABA neurons reported previously that exhibited inhibition to reward (Gallegos et al. 1999; Steffensen et al. 2001) were actually dopaminergic, since some neurons could potentially have been misclassified on the basis of their electrophysiological characteristics. Second, although it has been demonstrated that dopamine neurons respond with a phasic excitatory burst of spikes at *detection* of unpredicted reward, it has not been fully tested whether dopamine neurons would respond throughout a period of reward *consumption* since reward delivery is typically very brief (usually a single juice drop) (Schultz 1998). Therefore, we are unable to directly compare our 'tonic' outcome responses with the dopamine reward responses previously reported.

We found that neurons recorded in dopamine-rich areas of the midbrain responded with short-latency, phasic, excitatory bursts to presentation of reward-predictive stimuli in a manner that was similar to previously reported electrophysiologically-classified dopamine responses (Schultz 1998). Unlike earlier suggestions, we found that these responses did not correlate strongly with behavioural switching (Redgrave et al. 1999b). Additionally, we found that many neurons were inhibited during outcome consumption. Although these inhibitory reward responses appeared like those reported before from classified GABA neurons, we could not eliminate the possibility that at least some were from dopamine neurons. In summary, neurons in the dopamine-rich areas of the midbrain and in the nucleus accumbens (Wilson and Bowman 2004a, 2005) are predominantly excited to reward-predictive stimuli and inhibited during saccharin consumption.

# CHAPTER 6

## DEVELOPMENT OF A RAT BEHAVIOURAL TASK MEASURING REWARD "WANTING" AND "LIKING"

The work presented in this chapter is currently under review for publication in *Physiology & Behavior*.

**ABSTRACT**

It has been postulated that reward "wanting" and "liking" are mediated by separable brain systems. We developed a behavioural task with putative measures of reward "wanting" and "liking" available on a trial-by-trial basis. We were able to test whether our measures were sensitive to changes in thirsty rats' "wanting" and "liking" of liquid reward by manipulating its delay, taste and volume. We found that three of our putative "wanting" measures (anticipatory errors, reaction time and reward collection latency) and our putative "liking" measure (post-reward licking) were sensitive to variations in reward delay, taste or volume. The magnitude of effects were strongest for delay > taste > volume across measures of reward "wanting", whereas for our measure of reward "liking" taste=volume >> delay. Additionally, we investigated the impact of delay, taste and volume on our measures following acute, systemic administration of drug compounds that globally enhance serotonin and noradrenaline (imipramine), dopamine (GBR 12909) and opioid (morphine) function. Imipramine augmented the effects of delay and taste on reward "wanting", GBR 12909 attenuated the effects of delay on reward "wanting" and the effects of taste on reward "liking", and morphine reduced the effect of delay on a measure of reward "wanting". We propose that this task might be useful for future research into the specific neural mechanisms that contribute to the impact of reward parameters on "wanting" and "liking".

## INTRODUCTION

It has been postulated that reward "wanting" (motivation for a goal) and reward "liking" (hedonic appreciation of an achieved goal) are mediated by separable brain mechanisms (Berridge 2004; Berridge and Robinson 1998). We aimed to develop a behavioural task whereby automated measures of reward "wanting" and "liking" would be available on a trial-to-trial basis, allowing for future neurophysiological testing. To demonstrate our measures were sensitive to "wanting" and "liking" we manipulated the delay, taste and volume of liquid reward since these variables have previously been shown to alter rats' motivation (rats react quicker, wait longer and respond more for relatively immediate, large, and sweet rewards) (Brown and Bowman 1995; Hauber et al. 2000; Reilly 1999; Reynolds et al. 2002; Sclafani and Ackroff 2003; Spear and Katz 1991; Wakabayashi et al. 2004) and their appreciation of achieved reward (rats lick for longer for sweeter rewards) (Taha and Fields 2005).

Additionally, as a first step towards pharmacologically manipulating "wanting" and "liking" measures within this task, we tested rats following acute systemic administrations of drug compounds that globally increase serotonin and noradrenaline (imipramine hydrochloride), dopamine (GBR 12909 di-hydrochloride) and opioid (morphine sulfate) function. We predicted imipramine would interact with the effect of delay on reward "wanting", since imipramine can decrease 'impulsive' reward choices and 'impulsive' terminations of instrumental response chains (Bizot et al. 1988; Evenden 1998b). We postulated that GBR 12909 would interact with the effects of delay, taste and volume on reward "wanting" and of taste on reward "liking", since dopamine manipulations can affect impulsivity (Denk et al. 2005; Evenden 1998a; Evenden and Ryan 1996; Winstanley et al. 2003), dopamine neurons process predicted reward magnitude information (Fiorillo et al. 2003; Tobler et al. 2005), and elevations in dopamine can decrease the number of positive hedonic reactions during tasting of reward (Pecina et al. 2003; Wyvell and Berridge 2000). Finally, we hypothesized that

morphine would interact with the effects of taste and delay on reward "wanting" and of taste on reward "liking", since morphine can increase 'impulsive' reward choices, enhance reward consumption and increase the number of positive hedonic reactions during tasting of reward (Kieres et al. 2004; Rideout and Parker 1996; Zhang et al. 2003; Zhang et al. 1998; Zhang and Kelley 2002).

## METHODS

### SUBJECTS

Twelve Listar Hooded adult male rats (Harlan UK) weighed 375.5g (± 14.2g, 95% C.I.) when training began. These were housed in quadruplets on a light 12h: dark 12h light cycle. During training rats were placed on a regime of restricted water access with free access to water available from 4-5PM each weekday and from Friday 4PM until Sunday afternoon. During testing rats were given free access to water from 4-5PM everyday including weekends. Rats' body weights dipped no lower than 85% of their free-drinking weight. The "Handbook of Laboratory Animal Management and Welfare" (Wolfensohn and Lloyd 1998) was followed and all procedures conformed to the United Kingdom 1986 Animals (Scientific Procedures) Act.

### APPARATUS

Rats were trained in sound-attenuated testing chambers (34cm x 29cm x 25cm; Med Associates Inc., St Albans, VT) fitted with video cameras (Santec smart vision, model VCA 5156, Sanyo Video Vertrieb GmbH Co., Ahrensburg, Germany). Located on the centre of the left wall of each chamber was a drinking spigot, behind which a piezoelectric buzzer (model EW-223A, Med Associates Inc.) was concealed. On the right hand side of this wall was a nosepoke hole, within which were 3 identical blue/green LED's (luminosity~4.5mcd per LED) that could be programmed to be turned on and off independently. Two computer controlled syringe pumps (model PHM - 100, Med Associates Inc.) pumped liquids from 50ml glass syringes with stainless steel plungers (Rocket, London) through the drinking spigot at a rate of 0.05ml/sec. One of these syringes dispensed 0.5% w/v sodium saccharin solution and the other water. These solutions were delivered through separate lines of Teflon tubing and mixed on the rat's tongue. Solutions were delivered at reliable and precise times with standard

flow rates since the stiff syringes, plungers and tubing prevented pressure waves produced by the pumps from being attenuated.

## PROCEDURES

### *BEHAVIOURAL TRAINING*

Rats were trained on the following stages (N.B. Darija Hofmann and Emma Butler carried out most of the training procedures). The group was advanced to successive stages when their performance levels reached asymptote.

*Stage 1: Associating a conditioned stimulus with reward.* Rats were trained for 9 x 30min sessions to associate a conditioned stimulus (tone) with the availability of reward. Once the trial started (unsignaled to the rat) the rat made a lick at the reward spigot and, following a pre-conditioned stimulus delay of 0.1, 0.2, 0.4 or 0.8s, the conditioned stimulus was presented (each delay was randomly chosen from this list at the start of each trial and was not replaced until other delays had been used). Reward (0.05ml water lasting 1s) was delivered if the rat licked the spigot between 0.5s-2.5s after the onset of the conditioned stimulus. The conditioned stimulus was turned off at the end of reward delivery. The trial was ended (unsignaled to the rat) when the lick bout following the end of reward delivery had stopped (defined as an inter-lick interval >300ms). If the rats failed to lick during the presentation of the conditioned stimulus, a reward collection error was recorded and the next trial started.

*Stage 2: Nosepoke responding to earn reward.* Rats were trained for 14-22 30min sessions to make a nosepoke in the nosepoke hole to trigger the onset of the conditioned stimulus. Rats were randomly chosen to be in one of two groups to allow for future counterbalancing of cue light brightness with reward type (see stage 3). Depending on the group either 1 or 2 LED's (reward cue) were turned on within the nosepoke hole at the start of all trials. Rats had to maintain a nosepoke in this hole for a variable foreperiod (this value was chosen from a list

of 100, 300 or 500ms and was not replaced to the list until the other values had subsequently been chosen). The end of the foreperiod was signaled by the onset of the conditioned stimulus. Subsequent procedures were identical to those following conditioned stimulus onset in stage 1. If the rat failed to hold the nosepoke in the hole for the foreperiod delay then an 'early nosepoke withdrawal' was recorded; when rats failed to unpoke their nose from the hole within 4s of the conditioned stimulus onset a 'late withdrawal error' was recorded; instances where rats did not lick the spigot to trigger reward delivery within 15s of the conditioned stimulus onset, a reward collection error was recorded. Rats were returned to the start of the same trial following all errors.

*Stage 3: Associating reward cue brightness with reward quality.* Rats were trained for 31, 30min sessions to associate reward cue lights of varying brightness with different types of reward outcomes. There were four possible levels of cue brightness depending on the number of LED's illuminated within the nosepoke hole (0,1,2 or 3). One group of rats (n=6) received more preferable rewards (large saccharin solution (0.25ml, 0.5%w/v) > small saccharin solution (0.05ml, 0.5%w/v) > small water (0.05ml) > delayed small water (5s delay (see details below), 0.05ml water)) with increasing cue brightness, and the other group (n=6) with decreasing cue brightness. These four rewards allowed us to analyse putative effects of delayed discounting of reward (immediate small water *versus* delayed small water), reward taste (immediate small water *versus* immediate small saccharin) and reward volume (immediate small saccharin *versus* immediate large saccharin) on various behavioural measures.

At the start of each trial the reward type and foreperiod delay for that trial were chosen from a list containing all reward types at each foreperiod (this selection could not be chosen again until all the trial types in the list had been used). After the first training session we were unsure that the rats had time to decode the reward cues since early withdrawal errors were high and reaction times low. Therefore, we changed the foreperiod delays from 100, 300 and 500ms (used in

stage 2) to 300, 500 and 700ms. Except trials ending in delayed water reward, subsequent procedures were identical to those outlined in stage 2 (see Figure 6.1). In delayed water trials a 5s reward delivery delay was initiated following the first spigot lick made during conditioned stimulus presentation. On the basis of a model of discounting of delayed reinforcement in the rat, this delayed reward was approximately 20% of its immediate value (Reynolds et al. 2002).

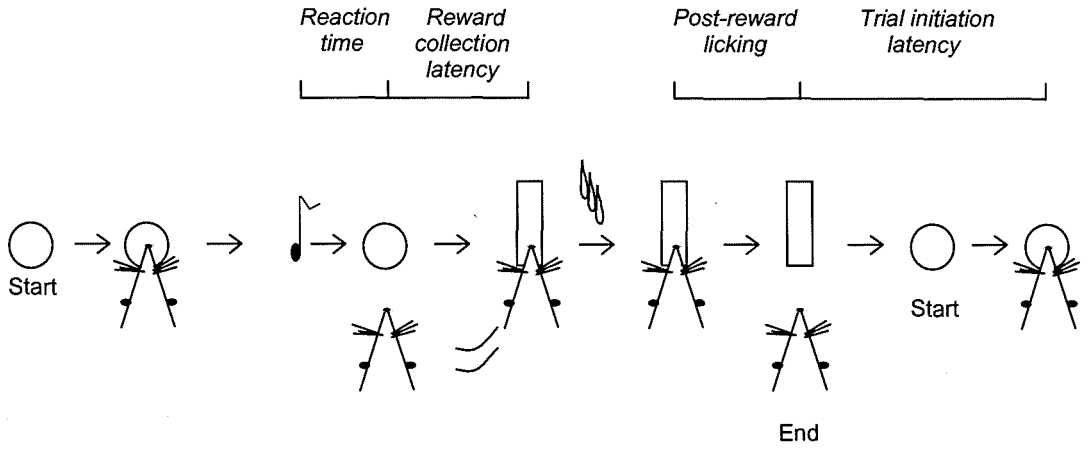



*Figure 6.1* Schematic representation of a rat performing a typical trial within the reward-value task and the behavioural measures recorded. Left-to-right: Circle shape represents nosepoke hole lit by cue lights. Musical note represents tone onset (conditioned stimulus), which remained on until the end of reward delivery. Rectangular shape represents reward spigot and liquid drops reward delivery. Behavioural measures: *Latency to initiate trial*: Time from trial start (cue light on in nosepoke hole) to nosepoke onset; *Anticipatory errors:* Number of trials per session rats withdrew their nose from the nosepoke hole before the onset of the conditioned stimulus; *Reaction time:* Time taken for rats to remove nose from nosepoke hole following conditioned stimulus onset; *Reward collection latency:* Time taken for rats to lick the spigot to trigger reward delivery after removing their nose from the nosepoke hole; *Post-reward licking:* Duration of post-reward lick bout (lick bout was completed when there an inter-lick interval>300ms, following reward offset).

*BEHAVIOURAL TESTING*

Rats were tested on the task outlined in training stage 3 following independent, systemic administrations of drug compounds in the following order: imipramine hydrochloride, GBR 12909 diHCl and morphine sulfate salt pentahydrate. All

doses were administered systemically by intraperitoneal injection, 30min prior to behavioural testing. To ensure different drugs did not interact, rats were not tested for two months between different drug administrations, during which they were given free access to water. Within each block of drug administration rats were initially tested following administration of the vehicle dose and then with each of the three drug doses. Drug dose order was fully counterbalanced across rats. The first drug injection was made the day after the vehicle injection and subsequent drug injections at three-day intervals. In order to keep the rats' water intake equivalent between each dose, rats were trained on the task on all drug-free days (these data were not analysed).

*DRUGS*

Imipramine hydrochloride (Sigma-Aldrich Co., UK) dissolved in 0.9% w/v saline was administered at a volume of 1mg/ml at 1, 5 and 10mg/kg doses. GBR 12909 diHCl (Organon Laboratories Ltd, Lanarkshire, UK) dissolved in the vehicle (2-hydroxy-propyl-β-cyclodextrin in 0.9%w/v saline (123mg/ml)) was administered at a volume of 2mg/ml at 10, 20 and 30mg/kg doses. Morphine sulfate salt pentahydrate (Sigma-Aldrich Co., UK) dissolved in 0.9% w/v saline was administered at a volume of 1mg/ml at 1, 2 and 4mg/kg doses. All doses were calculated using the molecular weight from the salt. N.B. One rat failed to respond following the 30mg/kg dose of GBR 12909, and another following the 4mg/kg dose of morphine.

DATA ANALYSIS

*BASELINE*

On the basis of anticipated effects of reward delay, taste and volume on behavioural measures of reward "wanting" and "liking" we independently performed orthogonal planned comparisons between immediate small water *versus* delayed small water (delay effect), immediate small saccharin *versus* immediate small water (taste effect) and immediate large saccharin *versus*

immediate small saccharin (volume effect) rewards across the five behavioural measures: trial initiation latency, anticipatory errors, reaction time, reward collection latency and duration of post-reward licking (see Figure 6.1). In order to correct for family-wise error across the tests for each behavioural measure, we calculated a corrected p value = 0.017 using the formula $0.05=1-(1-\alpha)^n$, where $\alpha$ corresponds to the corrected p value per comparison and n the number of comparisons (here, n=3). In cases when $p<0.017$ we decided we could reject the null hypothesis, whereas when $0.017<p<0.05$, we considered the effects as trends requiring future replication.

*DRUG EFFECTS*

We wanted to assess the impact of drug administrations on the effects of reward delay, taste and volume across the behavioural measures of reward "wanting" and "liking". We performed independent ANCOVAs to assess the relationship between drug dose concentration (covariate, *Dose*) and the difference scores between immediate small water – delayed small water trials, immediate small saccharin – immediate small water trials and immediate large saccharin – immediate small saccharin trials, independent of individual rat differences (fixed factor, *Rat*) across each behavioural measure (dependent variable). A significant effect of drug administration on the impact of reward delay, taste or volume on a particular behavioural measure was defined by a Main effect of *Dose* ($p<0.05$). The correlation coefficient *B* provided an estimate of the change in effect of reward delay, taste or volume on the dependent variable that could be attributed to the change in drug dose. All data were analysed using Microsoft Excel 2000™ and SPSS 10.0 for Windows™.

## RESULTS

### BASELINE TESTING

We identified trial initiation latency, anticipatory errors, reaction time and reward collection latency as putative measures of reward "wanting", since they occurred prior to the upcoming reward. In contrast, we identified post-reward licking as a putative measure of reward "liking", given that it had no positive bearing on the rat achieving reward. We found three of these putative measures of reward "wanting" were sensitive to the effects of reward delay or taste (see Figure 6.2). Thus, rats made fewer anticipatory errors when the upcoming reward was of preferable taste (trend effect) and they had quicker reaction times (trend effect) and reward collection latencies when the upcoming reward was to be delivered immediately *versus* delayed, (thereby delaying the reward even further). Additionally, we found that the putative measure of reward "liking", post-reward licking, was sensitive to the effects of reward taste and volume, since rats licked for longer following delivery of sweeter and larger reward (see Figure 6.2). The magnitude of effects were relatively stronger for reward delay > taste > volume on reward "wanting" measures, whereas reward taste and volume produced comparably strong effects on our measure of reward "liking", with reward delay having little effect (see Figure 6.3).

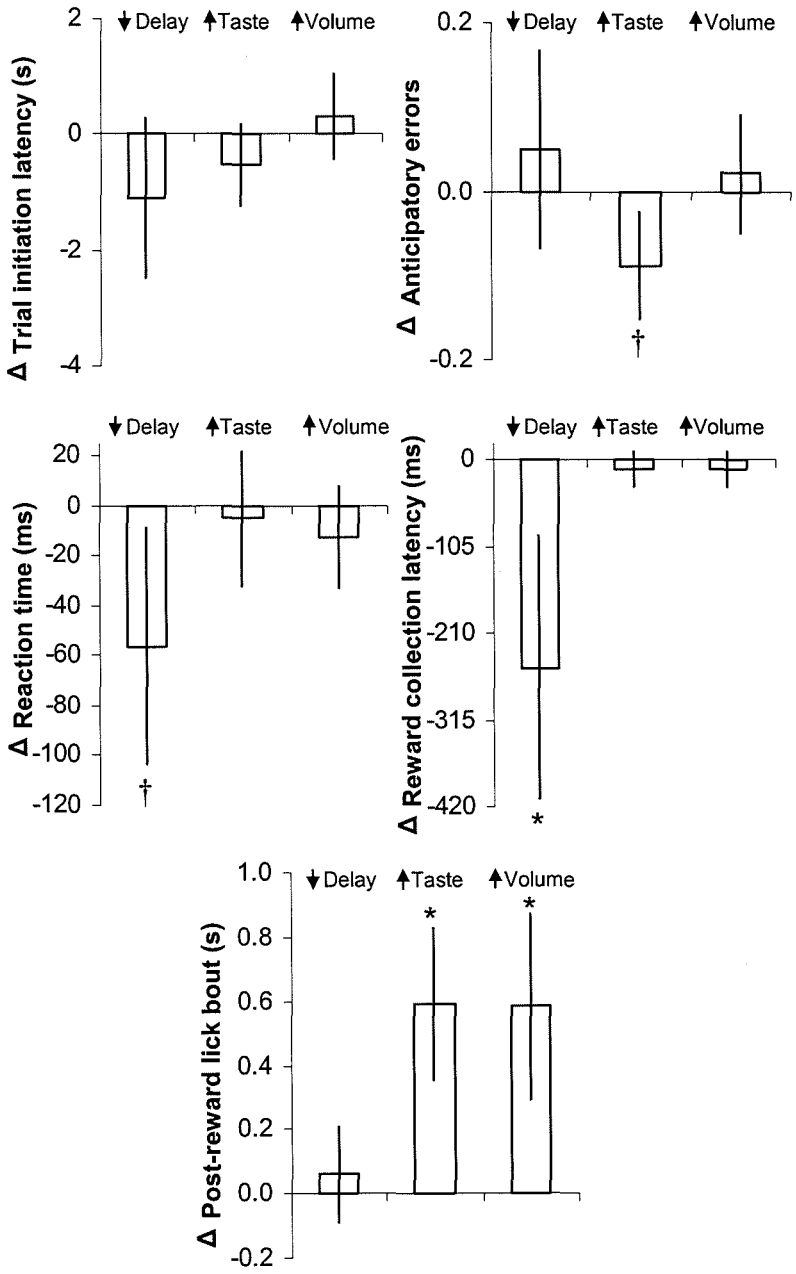



*Figure 6.2* See legend on next page

*Figure 6.2 (previous page)* Graphs indicating the mean difference scores across rats (n=12; ±95% CI) of immediate small water – delayed small water reward (Delay condition), immediate small saccharin – immediate small water reward (Taste condition) and immediate large saccharin – immediate small saccharin (Volume condition) across five behavioural measures (trial initiation latency (*top left*), anticipatory errors (*top right*), reaction time (*middle left*), reward collection latency (*middle right*), post-reward licking duration (*bottom*)). NB † indicates a trend effect following planned comparisons ($p<0.05$ but $> 0.017$, the p value corrected for family-wise error); * indicates a significant effect following planned comparisons ($p< 0.017$). Planned comparisons revealed that there was a tendency for rats to make fewer anticipatory errors in anticipation of upcoming immediate small saccharin *versus* immediate small water reward (*top right*; effect of taste: $F_{(1,11)}=6.734$, $p=0.025$, $\eta^2=0.380$) and a tendency for rats to react slower to the tone predictive of the upcoming delayed small water *versus* immediate small water reward (*middle left*; effect of delay: $F_{(1,11)}=5.444$, $p=0.040$, $\eta^2=0.331$). The rats were significantly slower to move from the nosepoke hole to the spigot to trigger reward delivery in anticipation of delayed small water *versus* immediate small water reward (*middle right*; effect of delay: $F_{(1,11)}=9.518$, $p=0.010$, $\eta^2=0.464$). Rats licked for significantly longer following delivery of immediate small saccharin *versus* immediate small water (*bottom*; effect of taste: $F_{(1,11)}=24.040$, $p<0.001$, $\eta^2=0.686$) and delivery of immediate large saccharin *versus* immediate small saccharin (*bottom*; effect of volume: $F_{(1,11)}=15.612$, $p=0.002$, $\eta^2=0.587$).

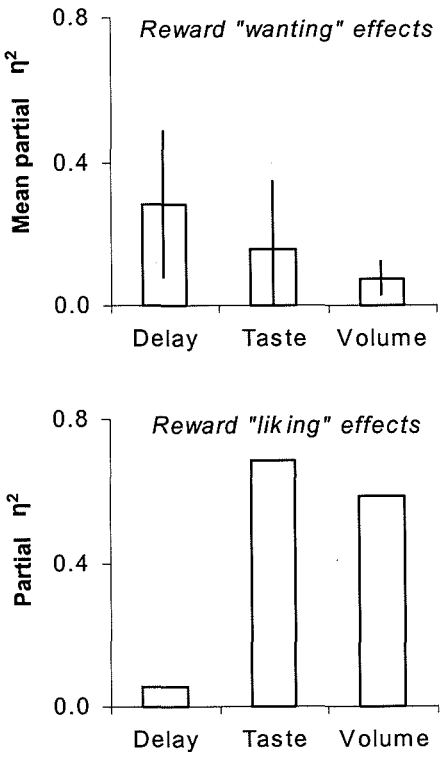



*Figure 6.3* Average magnitude of effects (partial $\eta^2$; ±95% CI) of reward delay (immediate small water – delayed small water), taste (immediate small saccharin – immediate small water) and volume (immediate large saccharin – immediate small saccharin) across the three behavioural measures of reward "wanting" (*top*; anticipatory errors, reaction time, reward collection latency) and single behavioural measure of reward "liking" (*bottom*; post-reward licking).

*IMIPRAMINE*

Imipramine interacted with the effect of delay on reward "wanting". Thus, as the dose of imipramine increased, trial initiation latencies differed more between immediate *versus* delayed and between sweet *versus* neutral tasting rewards. Similarly, reaction times became more differential between sweet *versus* neutral tasting reward, with increasing imipramine dose (see Figure 6.4).

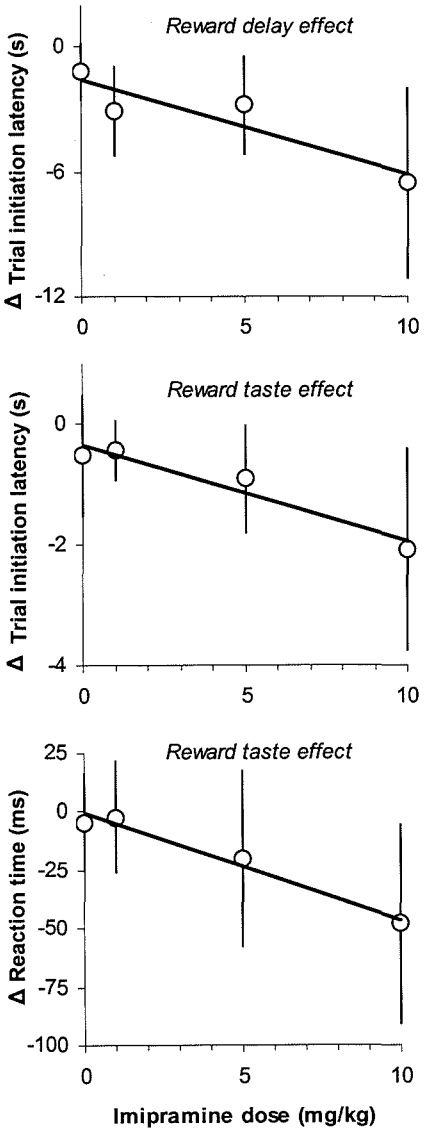



*Figure 6.4* Plots of mean difference scores across rats (n=12; ±95% CI) of trial initiation latencies for upcoming immediate small water – delayed small water reward (*top*), trial initiation latencies for upcoming immediate small saccharin – immediate small water reward (*middle*) and reaction times for upcoming immediate small saccharin – immediate small water reward (*bottom*) following administration of vehicle and imipramine (1, 5 and 10mg/kg). (*Top*) ANCOVA revealed a significant *Dose* effect ($F_{(1,35)}$=9.116, *p=0.005*, $\eta^2$=0.207) and a slope of *B*= -0.450. The pattern of effect indicates that with increasing imipramine doses, the effect of delay on trial initiation latencies was enhanced; (*Middle*) ANCOVA revealed a significant *Dose* effect ($F_{(1,35)}$=6.110, *p=0.018*, $\eta^2$=0.149) and a slope of *B*= -0.162. The pattern of effect indicates that with increasing imipramine doses, the effect of taste on trial initiation latencies was enhanced; (*Bottom*) ANCOVA revealed a significant *Dose* effect ($F_{(1,35)}$=6.081, *p=0.019*, $\eta^2$=0.148) and a slope of *B*= -4.556. The pattern of effect indicates that with increasing imipramine doses the effect of taste on reaction times was enhanced.

*GBR 12909*

GBR 12909 interacted with the effect of delay on reward "wanting" and the effect of taste on reward "liking". Thus, as the dose of GBR 12909 increased, rats' trial initiation latencies became more similar between immediate *versus* delayed reward and post-reward licking durations became more similar between sweet *versus* neutral tasting reward (see Figure 6.5). However, we did not find that GBR 12909 interacted with taste or volume on reward "wanting" or with volume on reward "liking", as initially predicted.

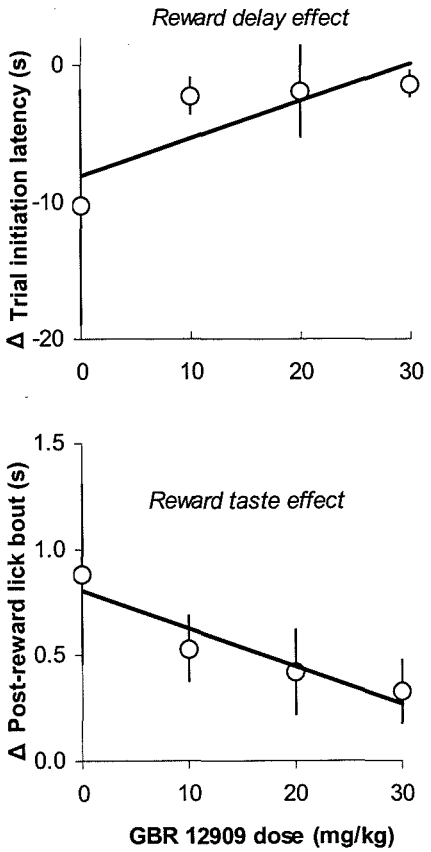



*Figure 6.5* Plots of mean difference scores across rats (n=11; ±95% CI) of trial initiation latencies for upcoming immediate small water – delayed small water reward (*top*) and the duration of the post-reward lick bout following delivery of immediate small saccharin – immediate small water reward (*bottom*) following administration of vehicle and GBR 12909 (10, 20 and 30mg/kg). (*Top*) ANCOVA revealed a significant *Dose* effect ($F_{(1,32)}$=6.799, *p=0.014*, $\eta^2$=0.175) and a slope of *B*= 0.272. The pattern of effect indicates that with increasing GBR 12909 doses, the effect of delay on trial initiation latencies was attenuated; (*Bottom*) ANCOVA revealed a significant *Dose* effect ($F_{(1,32)}$=8.557, *p=0.006*, $\eta^2$=0.211) and a slope of *B*= -0.018. The pattern of effect indicates that with increasing GBR 12909 doses, the effect of taste on the duration of the post-reward lick bout was attenuated.

*MORPHINE*

Morphine interacted with the effect of delay on reward "wanting". Thus, as the dose of morphine increased, there was less disparity between rats' trial initiation latencies for immediate *versus* delayed reward (see Figure 6.6). However, in contrast to our original predictions, morphine did not interact with taste on reward "wanting" or "liking".

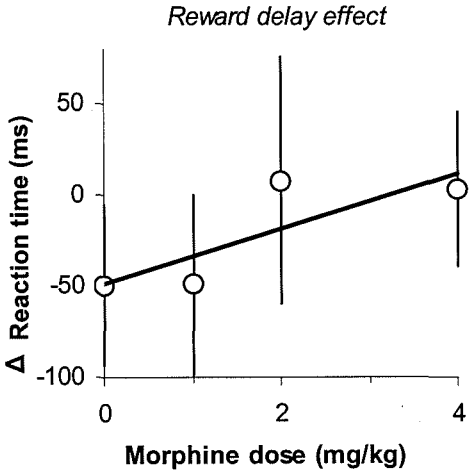



*Figure 6.6* Plot of the mean difference score across rats (n=9; ±95% CI) of reaction times for upcoming immediate small water – delayed small water reward following administration of vehicle and morphine (1, 2 and 4mg/kg). ANCOVA revealed a significant *Dose* effect ($F_{(1,26)}$=6.081, *p=0.019*, $\eta^2$=0.148) and a slope of *B*= -4.556. The pattern of effect indicates that with increasing morphine doses, the effect of delay on reaction times was attenuated.

## DISCUSSION

### SUMMARY

We developed a behavioural task within which rats worked for rewards that varied in delay, taste and volume. Baseline testing revealed that three measures (anticipatory errors, reaction time, reward collection latency) reflected reward "wanting" and one measure, reward "liking" (post-reward licking), since they were sensitive to changes in reward delay, taste or volume. We found that the average magnitude of effects were greatest for delay > taste > volume on reward "wanting" measures, whereas taste and volume had comparably strong effects on the reward "liking" measure. We additionally attempted to manipulate pharmacologically reward "wanting" and "liking" by independent acute, systemic, administrations of three drug compounds. We found that imipramine interacted with delay and taste on reward "wanting" with no effect on reward "liking", GBR 12909 interacted with delay on reward "wanting" and taste on reward "liking", and morphine interacted with delay on reward "wanting" with no effect on reward "liking".

### BASELINE MEASURES OF "WANTING" AND "LIKING"

Baseline testing revealed that 4/5 of our putative measures of "wanting" or "liking" were sensitive to manipulations in reward delay, taste or volume. However, in two of these cases (anticipatory errors and reaction times) the effects described were only statistical trends. Furthermore, there was no effect of delay, taste or volume on one of our measures (trial initiation latency) during baseline conditions. Thus, it is possible that we lacked statistical power to obtain statistically significant weak effects during baseline conditions. Indeed, drug manipulations seemed to produce the conditions necessary to obtain significant effects of delay and taste on trial initiation latency. Additionally, we found no effect of reward volume on reward "wanting" measures, yet this has been shown previously to impact upon

reaction times, waiting times and response rates (Brown and Bowman 1995; Hauber et al. 2000; Spear and Katz 1991). Therefore, for future replication of this task we propose that more subjects be tested to increase statistical power, and the volume of the immediate, large saccharin reward should be increased to try to equate the effect magnitudes of delay, taste and volume.

## DRUG ADMINISTRATIONS

### *GENERAL*

Since we analysed difference scores, we could only determine the relative impact of a given drug on two reward conditions. However, our measures meant that any general motor effects did not confound the pattern of results reported here.

### *EFFECTS OF IMIPRAMINE*

Previous work has demonstrated that imipramine attenuates the normally negative impact of delay on reward "wanting" (Bizot et al. 1988; Evenden 1998b). However, we found that imipramine exaggerated the effects of delay. Thus, it is possible that reward delay manipulations in choice (Bizot et al. 1988; Evenden 1998b) *versus* no-choice (as used here) paradigms are fundamentally different. Additionally, we found that imipramine enhanced the effect of taste on two measures of reward "wanting". This indicates that imipramine might increase "wanting" of sweeter rewards or alternatively decrease "wanting" of rewards with neutral taste. Either way, we have demonstrated for the first time that imipramine changes the impact of predicted taste on reward "wanting".

### *EFFECTS OF GBR 12909*

We found that GBR 12909 attenuated the effects of reward delay either by decreasing "wanting" for immediate small water reward or increasing "wanting" for delayed small water reward. We postulate that GBR 12909 did the latter, since enhanced dopamine neurotransmission typically increases reward "wanting" (Pecina et al. 2003; Wyvell and Berridge 2000). These data seem

consistent with previous demonstrations that dopamine enhancement increases impulsivity (Evenden 1998a; Evenden and Ryan 1996), although in this task there was no measure of impulsive choice. Somewhat surprisingly, although dopamine neurons respond phasically to encode predicted reward value and magnitude (Fiorillo et al. 2003; Tobler et al. 2005), global increases in extracellular dopamine by GBR 12909 did not interact with reward taste or magnitude. However, these data are consistent with previous reports that lesions or transient inactivation of the nucleus accumbens (which receives large dopaminergic input) and microinfusion of a dopamine D2 receptor (haloperidol) into the nucleus accumbens do not change rats' reaction times to discriminative cues predictive of rewards of different magnitudes (Brown and Bowman 1995; Giertler et al. 2003, 2004; Hauber et al. 2000). Finally, we found that GBR 12909 decreased the impact of taste on the duration of post-reward licking, consistent with demonstrations that increased dopamine neurotransmission can decrease the number of positive facial reactions during reward tasting (Pecina et al. 2003; Wyvell and Berridge 2000).

*EFFECTS OF MORPHINE*

Although morphine can make rats choose rewards more impulsively (Kieres et al. 2004), our data demonstrate that reward delay in a no-choice paradigm has *less* impact on reward "wanting" (as measured by trial initiation latencies) following morphine administration. These results parallel the effects of GBR 12909, both of which were opposite to the effects of imipramine. Therefore, it is possible that the serotonin system modulates the effects of reward delay in our task differently from that of the opioid and dopamine systems. In contrast to our predictions, we found no interaction between morphine and taste on measures of "wanting" or "liking". It should be noted that these negative results do not provide evidence that morphine does not in some way impact upon reward taste processing as has been hypothesized (Berridge 2003; Kelley et al. 2002). However, these data are perhaps consistent with previous findings that naltrexone (an opioid antagonist) does not reduce rats' initial licking rate of saccharin-sucrose solution from a bottle

(Frisina and Sclafani 2002).

## CONCLUSIONS

The behavioural task described here provides trial-by-trial, automated measures of reward "wanting" and "liking", which are modulated by reward delay, taste and volume. We found that global pharmacological manipulations impacted on some aspects of reward "wanting" and "liking" in the manner predicted. However, we failed to find many of the predicted effects, possibly in part due to low statistical power. Future studies might employ this task with modifications (increased sample size and reward volume) to try and understand the specific neural mechanisms contributing towards rats' "wanting" and "liking" of rewards with differing delay, taste and volume.

# CHAPTER 7

# GENERAL DISCUSSION

## SUMMARY OF EXPERIMENTAL FINDINGS

Initially, we asked questions regarding the functions of the mesoaccumbens dopamine system. We aimed to answer them by recording the activity of single neurons in the nucleus accumbens and in midbrain dopamine areas whilst rats responded within behavioural tasks. In the first set of experiments our main question was: do nucleus accumbens neurons respond equivalently to conditioned reinforcers and primary reinforcers? To answer this, we recorded from nucleus accumbens neurons in rats bar-pressing on a second-order schedule of saccharin reinforcement. In this task, presentations of second-order stimuli (conditioned reinforcers) only reinforced the temporal pattern of individual bar presses (Chapter 2). We found that nucleus accumbens neurons typically responded with opposite valence to conditioned reinforcers (excitation) *versus* primary reinforcers (inhibition; Chapter 3).

We wanted to investigate these types of responses to reward-predictive stimuli further. As has been hypothesized recently, it is possible that these kinds of responses cause the rat to switch it's behaviour from bar-pressing to reward consumption (Nicola et al. 2004b). To test this, we developed a task within which rats responded to conditioned stimuli predictive of outcomes of differential motivational valence (rewarding *versus* aversive) and caused a switch or 'no-switch' in the rat's behaviour. We found that nucleus accumbens neurons responded primarily to the outcome-predictive (usually *reward*-predictive) properties of conditioned stimuli rather than to their behavioural switching properties (Chapter 4). We found a similar pattern of responses from neurons within dopamine-rich areas of the midbrain (Chapter 5). This suggests that many neurons in the nucleus accumbens and dopamine-rich areas of the midbrain process outcome-prediction without encoding behavioural switching. Finally, we developed a behavioural task (Chapter 6) to allow for future investigation into the effects of reward delay, taste and magnitude on neural responses to reward-

predictive stimuli and reward consumption by neurons in the nucleus accumbens or midbrain dopamine areas.

## POSSIBLE FUNCTIONS OF NEURAL RESPONSES IN THE NUCLEUS ACCUMBENS AND MIDBRAIN DOPAMINE AREAS TO CONDITIONED STIMULI

### RESPONSE PATTERNS ARE INCONSISTENT WITH SEVERAL HYPOTHESES

We found that neurons in the nucleus accumbens (and dopamine-rich areas of the midbrain) typically responded with excitation to reward-predictive stimuli and/or inhibition during reward delivery (Chapters 3-5). It is possible that neurons responding to both the conditioned stimulus and reward with differential valence perform two independent functions (encoded by excitation and inhibition) on different efferent neurons. However, it is more parsimonious to assume that these neurons perform a single modulating function (by increases and decreases in action potentials) on a given efferent neuron. If the latter hypothesis is the case, we can rule out the following two potential explanations regarding the function of these sets of neurons. First, it is unlikely that these neurons encoded arousal *per se*, since both conditioned stimuli and reward were sufficiently arousing to modify the rat's behaviour. Second, it is unlikely that nucleus accumbens neural responses acted to reinforce preceding actions or response sequences made by the rat, since both conditioned and primary reinforcers in the second-order schedule reinforced the temporal pattern of responding similarly yet they often evoked neural responses of opposite valence (Chapter 2).

In chapters 4 and 5 we demonstrated that neurons in the nucleus accumbens and dopamine-rich areas of the midbrain responded predominantly to the outcome-predictive (primarily *reward*-predictive) properties rather than the behavioural switching properties of conditioned stimuli. Therefore, we have shown that these responses are unlikely to provide a global switching signal to switch behaviour as has been suggested (Redgrave et al. 1999b). However, there is evidence that manipulations in systemic dopamine (Bakshi and Kelley 1991a, b; Cools 1980; Evenden 2002; Evenden and Robbins 1983b), mesoaccumbens dopamine (Evenden and Carli 1985; Robbins and Koob 1980;

van den Bos and Cools 2003), and nucleus accumbens neurons (Bowman and Brown 1998; Reading and Dunnett 1991; Reading et al. 1991), can modulate switching between goal-directed behaviours. Indeed, we found that the activity of a minority of conditioned stimulus-responsive neurons in the nucleus accumbens and dopamine-rich areas of the midbrain did correlate with switching, typically to a variety of switching-outcome associations. However, this pattern of results is perhaps most indicative of movement-related neural activity.

We demonstrated that it is unlikely that neurons in the nucleus accumbens acted to promote the selection of particular actions since activity was not correlated with individual licks or bar-presses and since the average population response to conditioned stimuli did not differentiate between subsequent actions (Chapters 3 and 4). It has been recently found that the average response of a sampled population of nucleus accumbens neurons to a discriminative stimulus was strongest when the stimulus was followed by a nosepoke response made to earn reward (Nicola et al. 2004b). However, it is feasible that in trials containing the nosepoke response the discriminative stimulus had been more salient or motivating than in trials containing no nosepoke response, which might have caused a stronger 'reward-predictive' or 'goal-seeking' neural response. Therefore, neurons could have responded to promote general goal-seeking behaviours, rather than select the particular nosepoke action.

Although we did not analyse whether responses of neurons in dopamine-rich areas of the midbrain correlated with action selection, it has been demonstrated previously that the activity of dopamine neurons do not trigger particular actions (Ljungberg et al. 1992; Schultz and Romo 1990). Moreover, the population response of neurons responding to the reward-predictive conditioned stimulus in our go/no-go task was of greater magnitude to conditioned stimulus-1 *versus* conditioned stimulus-2, yet there were more conditioned responses after conditioned stimulus-2 than conditioned stimulus-1 (Chapter 5). Indeed, this pattern of responses is consistent with the hypothesis that post-learning,

responses are strongest to the stimulus that is the earliest predictor of reward (Hollerman and Schultz 1998; Schultz et al. 1993).

### RESPONSES ARE MOST CONSISTENT WITH THE REWARD-PREDICTION ERROR HYPOTHESIS

Unfortunately, we were unable to fully test whether responses by neurons in the nucleus accumbens or dopamine-rich areas of the midbrain encoded motivational salience *versus* motivational valence, since rewarding and aversive stimuli were not equally salient. However, we were able to assess whether neurons responded with opposite sign to stimuli that were of differential motivational valence. Although we found that nucleus accumbens neurons were predominantly excited to reward-predictive and aversive-predictive stimuli, neurons that responded to aversive-predictive *and* reward-predictive stimuli typically did so with opposite sign, consistent with findings from a similar recent study (Roitman et al. 2005). Indeed, it has been reported that the fMRI BOLD signal within the nucleus accumbens was modulated in a manner consistent with motivational valence in relation to reward prediction: activity was increased to unpredicted reward and decreased to omission of a temporally predicted reward (McClure et al. 2003).

Similarly, we found that single-neuron responses of in dopamine-rich areas of the midbrain were typically of opposite valence to reward-predictive (excitation) *versus* aversive-predictive (inhibition) conditioned stimuli, as was the average population response. It has previously been reported that dopamine neurons typically responded with differential valence to reward-predictive *versus* aversive-predictive conditioned stimuli in the macaque (Mirenowicz and Schultz 1996). Moreover, extracellular dopamine levels in the rat nucleus accumbens shell area was increased by delivery of unpredicted sweet food rewards, whereas it was decreased by delivery of unpredicted aversive-tasting quinine, and during aversive tail-pinch (Bassareo et al. 2002; Di Chiara 2002). Thus, our data are most consistent with reports from previous researchers that nucleus accumbens

and dopamine neurons can be differentially activated by reward-predictive *versus* aversive-predictive stimuli.

However, there are many reports that the nucleus accumbens and dopamine neurons respond equivalently to reward-predictive *versus* aversive-predictive stimuli, as if to encode motivational salience. For instance, fMRI BOLD signal in the nucleus accumbens was increased during delivery and anticipation of a painful or unpleasant stimulus and during expectation and delivery of rewarding stimuli (Becerra et al. 2001; Breiter et al. 2001; Breiter et al. 1997; Elliott et al. 2003; Jensen et al. 2003; O'Doherty et al. 2004). Moreover, extracellular dopamine is phasically released in the nucleus accumbens core during presentation of both rewarding and aversive tastes (Bassareo et al. 2002). Consequently, Di Chiara (2002) has proposed that extracellular dopamine release in the nucleus accumbens correlates with motivational valence in the shell and motivational salience in the core.

Thus, current evidence suggests that there are subsets of dopamine and nucleus accumbens neurons encoding motivational valence and others encoding salience. Why is there little neurophysiological evidence that dopamine neurons signal motivational salience? First, a subset of dopamine neurons might respond to motivationally stimuli that do not fulfill the electrophysiological criteria typically used to identify dopamine neurons. Indeed, we found neurons from the midbrain dopamine area that responded to outcome-switch combinations that were excited to aversive-predictive stimuli (Chapter 5). Second, aversive-predictive stimuli tested during dopamine recordings (air puff to the eye, taste of quinine or hypertonic saline) (Chapter 5; Mirenowicz and Schultz 1996) might signal the absence of reward (causing inhibitions) or the start of a new trial with the renewed possibility for the animal of upcoming reward (causing excitations). Moreover, it has been suggested that presentation of stimuli predictive of a strongly aversive consequence e.g. pain, are required to activate the mesoaccumbens dopamine system (Jensen et al. 2003). Thus, although there

might be dopamine neurons that encode salience, at a minimum there is a set of neurons in the dopamine-rich areas of the midbrain and nucleus accumbens that respond to motivational valence of stimuli in predicting the presence *versus* absence of reward.

The reward-prediction error hypothesis states that dopamine neurons encode the discrepancy between the actual and predicted occurrence of an incentive stimulus (Schultz 1998). Schultz further postulates that the nucleus accumbens might provide dopamine neurons with reward-predictive information. Unfortunately, since we recorded from neurons in the nucleus accumbens and dopamine-rich areas of the midbrain in different rats, we were unable to assess whether nucleus accumbens neurons encoded reward-prediction and midbrain responses encoded reward-prediction *error*. We were also unable to interpret whether responses in one area triggered responses in the other and vice versa since rats between studies had slightly different amounts of training.

However, from our data responses to conditioned stimuli were strikingly similar between the populations of neurons in the nucleus accumbens *versus* the dopamine-rich areas of the midbrain. Thus, it seems likely that both sets of neurons were contributed to the same function of reward-prediction. Moreover, given that there is an anatomical bias towards more connections from nucleus accumbens to midbrain dopamine neurons than vice versa (Haber et al. 2000; Joel and Weiner 2000), it seems feasible that in line with Schultz's predictions, some of the nucleus accumbens responses drove those in the midbrain. This hypothesis is additionally supported by evidence that reward-predictive information is processed by neurons projecting to the nucleus accumbens (orbitofrontal cortex, amygdala and hippocampus (Schoenbaum et al. 1999; Shibata et al. 2001)), whereas salience not reward-predictive information is processed by neurons in the pedunculopontine tegmental area, which directly project to dopamine neurons via a non-striatal pathway (Pan and Hyland 2005). Thus, our data are consistent with other recent data, suggesting that reward-

predictive information arising in cortex, amygdala or hippocampus might funnel through the nucleus accumbens to dopamine neurons.

## POSSIBLE FUNCTION OF RESPONSES DURING OUTCOME DELIVERY

We found that most responses during reward delivery by neurons in the nucleus accumbens and dopamine-rich areas of the midbrain were inhibitory. It is possible that these responses 'gated' reward consumption, contributed to reward "liking" or reflected a decrease (or resetting) of preceding excitatory activity during goal-seeking behaviour.

### RELATIONSHIP OF RESPONSES TO REWARD CONSUMPTION AND REWARD "LIKING"

It has recently been argued that inhibitions during outcome delivery in the nucleus accumbens encode reward consumption, not reward "liking" (Taha and Fields 2005). These authors found a subset of nucleus accumbens neurons that was typically inhibited during sucrose reward delivery but was also inhibited during lick bouts made in the absence of reward. Additionally, these neurons were not inhibited differentially by the relative sweetness of reward. Conversely, there was a different subset of neurons that were typically excited during sucrose delivery, which exhibited responses differentially to the relative taste preference of sucrose reward and did not respond during lick bouts made in the absence of reward delivery. The authors suggested that the former set of neurons might facilitate the onset of a feeding bout (consumption), whereas the latter set might encode palatability, presumably an aspect of reward "liking". Similarly, it has previously been reported that excitations by nucleus accumbens neurons in the macaque can differentiate between delivery of preferred rewards or rewards of differing magnitude (Cromwell and Schultz 2003; Hassani et al. 2001). Additionally, Taha and Fields (2005) propose that opioid agonism in the nucleus accumbens (previously implicated in contributing to palatability encoding (Kelley 2004; Kelley et al. 2002)) might inhibit striatal interneurons causing disinhibition (relative excitation) of nucleus accumbens neurons during reward palatability encoding.

However, in our experiments, if bi-valent activity by single neurons serves a single function, and if inhibitions were to facilitate consummatory responses then excitatory responses to conditioned stimuli/conditioned reinforcers would have to correlate with a reduction in consummatory behaviours. However, we found that nucleus accumbens neurons responded with opposite valence to conditioned reinforcers (or conditioned stimuli) and primary reinforcers, both of which stimulate consummatory behaviour (Chapters 3 and 4). Thus, the inhibitory responses in our task are unlikely to have 'gated' consummatory behaviour as has been proposed (Taha and Fields 2005).

Could inhibitory responses instead have encoded aspects of reward "liking"? Although this is possible since opioid agonists predominantly inhibit nucleus accumbens neurons (Chang et al. 1998; Lee et al. 1999) and opioid-induced inhibition of nucleus accumbens neurons increases feeding for palatable rewards such as saccharin (Kelley 2004; Kelley et al. 2002), we have not explicitly tested this hypothesis on our neuronal samples. However, we aim to do so in future studies by recording from nucleus accumbens neurons and correlating their activity with measures of rats' "wanting" and "liking" of rewards with differing delay, taste and volume using the behavioural task described in Chapter 6.

### RESPONSES ARE MOST CONSISTENT WITH A DECREASE IN GOAL-SEEKING

We have provided arguments against the hypothesis that neurons in the nucleus accumbens in our experiments encoded reward consumption activity. Moreover, we have not tested the hypothesis that responses encoded reward "liking". We postulate that the most parsimonious explanation for the function of nucleus accumbens neurons in our experiments is that they encoded a goal-seeking signal. Thus, during goal-seeking neurons typically responded with excitation to stimuli predictive of an upcoming outcome. In contrast, during reward delivery, when rats no longer sought reward, inhibitions may have acted to reset preceding excitatory tonic or phasic 'seeking' activity. This might also explain why some

neurons were inhibited during unreinforced lick bouts (Taha and Fields 2005), since neurons might have been relatively excited prior to licking during goal-seeking behaviour and subsequently inhibited during the temporary cessation of goal-seeking behaviour whilst the rats licked.

Similarly, this hypothesis provides the best explanation for the inhibitory population responses by neurons in the dopamine-rich areas of the midbrain during outcome delivery (Chapter 5). Since the average population was inhibited during saccharin *and* quinine delivery, yet rats did not consume quinine (responses unlikely to encode consumption, *per se*), and they found saccharin, rewarding and quinine, aversive (responses unlikely to encode "liking"), it seems most likely that inhibitions encoded a reduction in preceding tonic or phasic excitatory, goal-seeking activity.

Although we found that nucleus accumbens neurons were predominantly inhibited during saccharin consumption, there was a greater proportion of inhibitory *versus* excitatory reward responses during saccharin delivery within the second-order schedule (91%; Chapter 3) than within the modified go/no-go task (65%; Chapter 4). There are many potential reasons for this including the length of delivery of saccharin (2s *versus* 4s), the length of time windows to assess the neural response (2s *versus* 4s), the extent reward was predicted by conditioned stimuli, the frequency with which reward was earned by the rat within a session, and random sampling biases.

## FUTURE RESEARCH

### REWARD-PREDICTION AND REWARD CONSUMPTION

The work presented here has led to many new questions regarding the function and mechanism of single-neuron activity within the mesoaccumbens dopamine system. Nucleus accumbens responses to conditioned stimuli in Chapter 5 were strikingly similar in appearance to dopamine responses previously reported in the macaque (Schultz 1998; Schultz et al. 1993), suggesting that they might interact to serve a similar function. Thus, given recent demonstrations that midbrain dopamine neurons encode the predicted value of upcoming reward (Fiorillo et al. 2003; Tobler et al. 2005), do nucleus accumbens neurons respond to predict the upcoming reward value? Moreover, could responses during reward delivery reflect the value of received reward? This could be tested by recording nucleus accumbens neural activity from rats responding within the behavioural task described in Chapter 6 devised to assess the effects of delay, taste and volume on measures of reward "wanting" and "liking". Additionally, it is possible that putative "liking" responses are modulated not only by reward taste but also by the relative value of reward in relation to the preceding work cost, a pattern of results which might be found when recording nucleus accumbens neurons whilst rats respond for rewards under a variety of work schedules.

There is accumulating evidence that different pharmacological functions (e.g. dopamine, GABA, AMPA and opioid) within the nucleus accumbens promote different types of behaviours with respect to "wanting", "liking" and feeding (Kelley 2004). Therefore, it is possible that during neural recording in the nucleus accumbens microinjection of different pharmacological compounds (e.g. GABA, AMPA or opioid agonists/antagonists) into the recorded area would reveal different clusters of neurons that react to particular pharmacological compounds and correlate with (or cause) particular sensory (e.g. conditioned stimulus response), psychological (e.g. reward "liking") or behavioural (e.g. licking) events.

We have demonstrated that there was no clear pattern of 'switching responses' in the nucleus accumbens or dopamine-rich areas of the midbrain. It is possible that these types of responses are present elsewhere in the brain. Given recent evidence that neurons in the pedunculopontine tegmental nucleus respond to the salient, rather than reward-predictive properties of conditioned stimuli (Pan and Hyland 2005), these neurons could potentially perform the switching function proposed by Redgrave et al. of dopamine neurons (Redgrave et al. 1999b). Alternatively, the switching mechanism could be performed across ensembles of neurons by selection and de-selection of different individual movement-related neurons or of different neuronal circuits that encode behavioural sequences. Therefore, it might be of interest to record the activity of single neurons in the dorsal striatum during the modified go/no-go task since these neurons can respond to particular goal-directed movements (Hollerman et al. 1998). Additionally, by recording single-neuron activity throughout different areas within striato-nigro-striatal and cortico-basal ganglia-thalamo-cortico circuits of individual animals, it might be found that attention and/or behaviour is switched by selection and de-selection of different loops, rather than of different neurons.

Another consideration for future research is to find a way to record with more certainty from *all* dopamine neurons within alert animals. Thus, although we did find some subtle responses were correlated with aspects of behavioural switching in dopamine-rich areas of the midbrain, we were unable to identify whether these neurons released dopamine in the nucleus accumbens. Indeed, some neurons that release dopamine or cause other neurons to release dopamine might respond to encode motivational salience, as recently proposed (Di Chiara 2002).

## POSSIBLE EVERYDAY EXPERIENCE OF THESE NEURAL RESPONSES

It is important to speculate on how responses from single neurons in the nucleus accumbens and midbrain dopamine areas of the rat contribute to the psychological processes people experience in everyday life, since this is one of the ultimate goals of the research. We have shown that nucleus accumbens neurons in the rat respond to stimuli that reinforce a chain of complex actions. These kinds of responses might stimulate people to work for money, which is a conditioned reinforcer for rewards such as food and shelter. It has been hypothesized that responses to outcome-predictive conditioned stimuli might facilitate learning of new goal-directed actions (Schultz 1998). This process could apply to basic motivated behaviours such as eating and drinking, as well as to highly complicated sequences of goal-directed actions such as playing football or video games. Moreover, it is possible that responses to reward-predictive and aversive-predictive stimuli in the mesoaccumbens dopamine system contribute to 'wanting' and 'avoiding' or 'excitement' and 'fear', respectively. In some situations, responses to reward-predictive stimuli might make people excessively "want" reward or reinforcers, resulting in addictions or obsessions.

## CONCLUSIONS

Our main findings are: (1) Second-order stimuli (conditioned reinforcers) presented within a second-order schedule of saccharin reinforcement reinforced the temporal pattern of behaviour but not overall bar-pressing rates. This has important implications for researchers using second-order schedules to assess the impact of anti-addiction drugs on drug-associated stimuli. (2) The activity of nucleus accumbens neurons did not act to reinforce preceding actions. In fact, they often responded with opposite valence to conditioned reinforcers and primary reinforcers. We suggest that nucleus accumbens neurons might encode a goal-seeking signal. (3) Many neurons in the nucleus accumbens and dopamine-rich areas of the midbrain responded to the outcome-predictive properties of conditioned stimuli and not to switch rats' behavioural strategy. (4) A behavioural task was developed in the rat to allow us to assess neural responses of nucleus accumbens and dopamine neurons during "wanting" and "liking" of rewards of different delay, taste and volume. In summary, it seems that neurons in the nucleus accumbens and dopamine-rich areas of the midbrain respond to the motivational value of conditioned stimuli and during reward consumption. Understanding more about the function of these responses is fundamental to discovering how our minds work.

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